An Infant in Swaddling Clothes This is probably done with the idea of [illegible] plaque by Andrea della Robbia (1 Florence By kind permission of

THE
ITAMINS IN MEDICINE

by

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PREFACE TO THE THIRD EDITION

In the preparation of the third edition of this book most of it has been ewritten This has been necessary owing to the changing state of our knowledge and the advances made in certain directions, notably the physiology and biochemistry of the vitamin B complex Although the number of references has been increased from about 4,500 to 5,500 and the illustrations from 208 to 245 the size of the book remains about the same

Our work has been greatly helped by suggestions and criticisms and also by the generosity of those who have allowed us to reproduce their original llustrations We are especially indebted to the many workers in England, he Continent and the Americas who besides sending us reprints of their ublished articles have kept us informed about their current research and s results

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CHAPTER I

VITAMIN A

THE ANTIXEROPHTHALMIC VITAMIN
THE ANTI INFECTIVE VITAMIN
AXEROPHTOL

VITAMIN A is the term used to include vitamin A_1 found in animals and sea fish, and vitamin A_2 found chiefly in fresh water fish Biologically and chemically they are so very similar that generally no distinction is made between them The little that is known about vitamin A_2 is discussed on p 85 Axerophtol was the name given by Karrer in 1938 to vitamin A_1, but it has not come into common use, except on the Continent

Provitamin A is the name given to those plant carotenoids which can be converted by animals to vitamin A

HISTORY

The most clear cut effect of lack of vitamin A is night blindness, which often occurs suddenly after long exposure to a day's bright sunlight In rural communities inability to see in the dusk is a very serious condition fishermen for instance, may walk off the rocks into the sea after landing in the evening Night blindness can be cured often in twelve hours, by eating food rich in vitamin A, such as liver The dramatic quickness both of the onset and the cure explains why liver has been used for centuries for night blindness

The Ebers Papyrus [1], written about 1600 B C, probably referred to night blindness when liver was recommended for the eyes, while the Chinese in 1500 B C were giving liver, honey, flying fox dung and tortoiseshell, all of which] Hippocrates advised the whole liver (as known to later Roman writers Jacob fourteenth century, may be thus translated [2],

> He who cannot see at night,
> Must eat the liver of the goat
> Then he can see all right

Guillemeau in France in the sixteenth century besides clearly describing night blindness, advised liver for its cure [3] which was also advised by other writers at that time [5]

Drummond and Wilbraham [2] find that the first mention of liver for the eyes in England was in Muffett's "Health's Improvement" (1655), though Bayly, at one time Queen Elizabeth's physician, in his book on eyes recommends "rawe herbes" among which is "eie bright", but the only evidence of night blindness being common at this time is references to mists and films over the eyes "Rawe herbes" would of course provide provitamin A

Aykroyd [1] in his accounts of Newfoundland and Labrador fishermen says they not only recognize how bright sunlight may bring on night blindness, but also use liver, preferably the raw liver of a gull or puffin, for a cure

The beginning of the present century saw the realization that more

serious eye affections—especially 'conjunctivitis' in children—were due to lack of some food factor. Mori [4] in Japan in 1904 treated juvenile conjunctivitis with cod liver oil, believing the diet was inadequate in fat, while Monrad in 1917 thought that the outbreak of conjunctivitis which occurred in Danish children at that time was due to a deficiency of a fat-soluble factor, caused by the export of the country's animal fats to England and Germany.

Animal experiments as early as 1909 had shown that rats on deficient diets developed conjunctivitis [6]; that a fat ... was proved by Osborne and Mendel [7] in 1913 ... in 1917. The latter workers called the fact... out the similarity between xerophthalmia in rats and the conjunctivitis found in children on fat deficient diets.

Conjunctivitis and xerophthalmia are, however, only the most noticeable examples of the change in the epithelial surfaces of the body brought about by lack of vitamin A. Wolbach and Howe [9] in 1925 found that the specific tissue change due to deprivation of fat soluble vitamin A is replacement of various epithelia by stratified squamous keratinizing epithelium. This replacement of a specialized epithelium by a primitive type leads to a lowered local resistance to infection.

The name 'anti-infective' vitamin was first given to vitamin A by Green and Mellanby [10] in 1928 because they found that animals killed by lack of vitamin A showed multiple foci of infection in those areas where the epithelium had altered. At this time the infection was regarded as the direct, and not secondary, effect of lack of vitamin A.

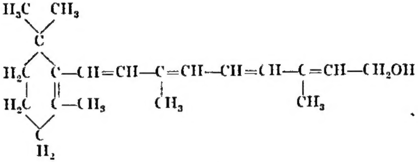
The separation of vitamin D from vitamin A was not complete before 1922. As early as 1909 Stepp [11] had found that there was some unrecognized factor in fats necessary for growth, and in 1913 McCollum and Davies [12] and also Osborne and Mendel [7] confirmed this, the latter workers also stressing that different fats varied in their value for growth. Mellanby in his work on rickets from 1918 onwards originally believed that the antirachitic factor, whose existence he discovered, was the same as the fat soluble A factor of McCollum and Davis. But in 1922 and the following years several very important papers appeared ... ng that there were two separate factors in ... anti-xerophthalmic factor and the anti-rac... also Goldblatt and Soames [14] found that ultra-violet irradiation, while it cured rickets, would not prevent xerophthalmia or maintain growth in animals on fat deficient diets. A year later in 1923 Goldblatt and Zilva [15] found that the growth promoting and antirachitic functions of cod liver oil were destroyed by heat and oxidation at different rates, and they also observed that spinach was excellent for growth but not for preventing rickets. Mellanby [16] in 1926, comparing the diets of a series of puppies which had died or survived an epidemic of bronchopneumonia, reported that the protective value of the diet against infection was not related to its protective value against rickets.

The carotene content of plants was found by Rosenheim and Drummond [17] in 1920 and by Coward [20] in 1923 to vary with their vitamin A potency, a relationship which was further emphasized by Rosenheim and Drummond's [18] observations in 1925 on the similarity of the colour reactions of the two [19]. Between 1929 and 1930 Moore [21, 22, 23] and Capper [24] were largely responsible for showing that carotene could be used by animals as a source of vitamin A, into which it is converted in the body.

The chemistry and isolation of vitamin A and its relationship to carotene was settled chiefly by the work of Karrer [25, 26, 27] and Heilbron [28, 29] and their co-workers and of Holmes and Corbett [30] between 1930 and 1937. The final synthesis [31] was the result of English, Dutch, Swiss and American research which, stimulated by the threat of a shortage of vitamin A during the Second World War, came to a successful conclusion in 1947.

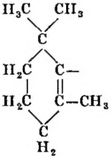
CHEMISTRY OF VITAMIN A AND CAROTENE

..arrer and his collaborators in Switzerland [25, 26, 27, 33, 34] first ggested the now accepted formula for vitamin A, which was confirmed by ilbron and others [28, 35] in England. Vitamin A has the formula,

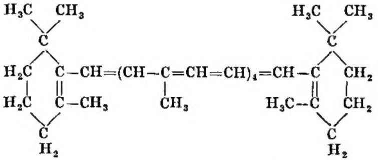


ing formed in the body from one of its carotenoid provitamins, alpha-, ta , and gamma-carotene and cryptoxanthine [32], and a few other rare rotenoids (p 10)

All the carotenoid provitamins have the same fundamental structure as tamin A, they possess the same essential unsubstituted beta-ionone icleus [34, 36]

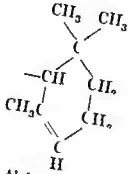


ith a similar aliphatic side chain, though the latter is twice as long with terminal group which gives to each carotenoid its own particular properties or instance, in beta carotene [25, 33]

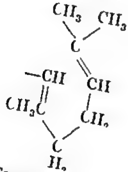


is terminal group is a second unsubstituted beta-ionone nucleus, and so e whole molecule could be in theory, and in the living animal most probably split by hydrolysis at the middle of the aliphatic chain into two complete olecules of vitamin A (p 13) even though the chemical conversion of ta carotene to vitamin A is extremely difficult and gives very small elds [37] The Russian statement that vitamin A is formed when carotene treated with iodinated casein is incorrect [38]

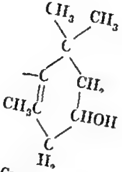
The other carotenoids not having a second unsubstituted beta ionone icleus as their terminal group,



Alpha carotene



Gamma carotene



Cryptoxanthin

but in its place the various terminal groups [39] shown above cannot form more than one molecule of vitamin A

Stereomeric isomers of both vitamin A and its carotenoid precu occur naturally In theory vitamin A—since it has but two effective double bonds [41]—can have only four different spatial config tions trans trans trans cis cis trans and cis cis Vitamin A is believe be the trans trans form and neovitamin A (p 6) the only other form vitamin A so far known to occur naturally

The carotenoid stereoisomers were first described by Gillam and Ridi [44] in 1936 when they found that during adsorption on alumi beta carotene was spontaneously transformed into pseudo alpha carotene —the neo beta carotene B of later workers Since 1936 much research ha been devoted to the carotene isomers but they are mostly artificial products almost all the naturally occurring carotene fortunately being in the all tran form so that for instance the thirty two possible alpha carotenes can remain a fascinating academic study without being a nightmare in practical nutrition The physiology of the carotenoids being discussed on p 10 it is only necessary here to emphasize that the tendency to spontaneous isomerization may complicate analyses involving carotenoids

Vitamin A and its carotenoid precursors being fat soluble and unsapon fiable are found concentrated in the unsaponifiable extracts of fats Separatio of vitamin A from the carotenoids can be carried out by dissolving both i petroleum ether and adding alcohol when the latter will dissolve the vitami A but not the carotenoids Since alcohol and petroleum ether are not miscible the alcohol layer containing the vitamin can be easily separated Vitamin A esters however remain with the carotenoids Chromatographic adsorption is extensively used for the separation of vitamin A from other substances such as kitol [50] for the separation of the alcohol from the ester [77] or for the separation of the carotenoids from each other [78]

Pure crystalline vitamin A alcohol was first isolated—from fish liver oil— by Holmes and Corbett [30] in 1937 though it was subsequently shown that the pale yellow crystals were solvated their true melting point not being 6° C but 60° C The blue value (p 6) was 100 000 and E $\frac{1\%}{1\text{ cm}}$ 328 equalled 2 000 The biological activity was reported to be 3 000 000 I U per gram which gave a conversion factor nearer 1 500 than 1 600 (p 9) Mead Underhill and Coward [45] in 1939 using two crystalline esters of vitamin reported that the biological activity was 3 181 000 and 3 424 000 I U gram and since E $\frac{1\%}{1\text{ cm}}$ 328 equalled 1 600 1 800 the conversion factor w about 2 000 Many subsequent workers have confirmed these findings [39 46] apart from very minor differences and the crystalline vitamin A acetat is now used in the U S A as a Reference Standard (p 10) Some American reports [46] about abnormally high biological values and conversion factors for the crystalline alcohol and ester were due to one of the older undervalued U S A Reference Oils having been used for the assays (p 10)

Three other forms of vitamin A besides vitamin A_2, which is discussed on p 85, may occur in fish liver and other oils anhydro or "cyclized"

Fig 1 Vitamin A—Alcohol crystals Magnification 10 ×

vitamin A kitol and neovitamin A The first of these [47] is formed spontaneously in fish liver oils which have been "maltreated", it is not a

Fig 2 Neovitamin A—Alcohol crystals Magnification 10 ×

cyclized vitamin A alcohol as was at one time thought but is a hydrocarbon formed by dehydration which has no biological activity Its only practical

importance is that it robs oils of some of their value and its absorption bands with the Carr-Price reaction may complicate assays by overlapping those due to vitamin A.

Kitol [48, 49, 50], on the other hand, is a di(vitamin A) normally present in large amounts in fresh whale-liver oil and has been reported in that of sharks, dogfish and lambs, while in that of pike is a substance similar to kitol but related to vitamin A_2. Kitol has no biological activity and causes considerable difficulty when estimating vitamin A in whole liver oils owing to its colour reactions, though it can be removed by chromatography [50]. Barua and Morton [50] have suggested that it may be formed to detoxify excessive accumulations of vitamin A. When heated it forms vitamin A and degradation products, a reaction of great value in increasing the yield of the vitamin.

Neovitamin A [51, 54] forms about thirty five per cent of the vitamin A in many common fish-liver oils. It is a stereoisomer of vitamin A, probably being in the *trans cis* form, and it has the same biological potency for rats, being converted into the usual *trans-trans* form and stored as this in the liver after feeding. It has maximum absorption in the ultra-violet region of 328 millimicrons, a conversion factor of 1645 and it reacts more slowly than vitamin A with maleic anhydride, this forming the basis of its assay in fish liver oils.

The synthesis of vitamin A became of practical importance during the Second World War because of the risk that supplies from fish liver oils could not be maintained. The consequent wave of research, besides showing that all earlier reported syntheses—such as that of Kuhn and Morris [40] in 1937—could not be confirmed, did also produce, in amounts too small to have any dietetic value, a number of substances with vitamin A activity; but Milas [41], writing in 1947, ended his very detailed review of the highly complicated chemistry of this subject by pointing out that "in no case has a synthetic product been shown to be identical in *every respect* with the natural product". However, by 1948 Sir Ian Heilbron [31], in a very lucid summary of current research, was able to talk of "the large scale production of the synthetic vitamin". For further progress the reader should consult the papers by Isler, Wendler or Milas and their collaborators [42] and that of Sobotka and Chanley [43] who give a preliminary account of research designed for the total synthesis of vitamin A without starting from the natural beta-ionone, mostly obtained from lemon grass oil, which all other workers have used.

Vitamin A gives a band of maximum absorption in the ultra-violet region at 325 millimicrons [39], but as it was originally thought [52] that the maximum absorption was at 328 millimicrons—which is now known to be due to the neovitamin A of fish liver oils discussed above—this is still generally used when determining vitamin A in fish liver oils by spectrophotometry [53].

The colour reactions of vitamin A are of great practical importance. They occur with sulphuric acid [17] and with the chlorides of polyvalent metals [18, 19], the most satisfactory reagent was found by Carr and Price [19] to be antimony trichloride dissolved in chloroform. This test is often called the Carr Price colour test. With vitamin A a blue colour develops rapidly and then fades. The blue colour, however, is not only given by vitamin A but also by carotenoids [18] and "oxycholesterol" [54]. Therefore to confirm the presence of vitamin A the absorption spectrum of the Carr-Price reaction must be examined, when, if vitamin A is present, specific absorption bands will be found. Activated glycerol dichlorohydrin has been used instead of antimony trichloride [55] but it would appear to have no advantages [56].

Vitamin A exhibits fluorescence when irradiated by a mercury vapour lamp [57]; this has been used by Popper to demonstrate vitamin A in the tissues (Figs. 3, 4, 5) by the technique which he describes in the *Archives of Pathology* [58]. The differences in the fluorescence of the alcohol and the

ester have been used to determine the amounts of each in preparations of vitamin A [57]

Biological activity is lost when vitamin A takes part in chemical reactions which affect double bonds such as oxidation hydrogenation and bromination [59] Heat does not destroy vitamin A in butter at temperatures below about 120° C in the absence of oxygen [60], when oxygen is present slow destruction occurs in oils even at room temperatures [61] and rancidity accelerates this though it probably causes no appreciable loss in human food [64] Many fats when heated develop an anti vitamin A factor which destroys the biological activity of vitamin A whether this is a chemical or biological effect is unknown [65 66] Exposure of cod liver oil to light reduces its vitamin A content so the oil should be stored in dark bottles [62 63] Vitamin A in cod liver oils prepared from concentrated fish liver oils diluted with cottonseed peanut or maize oil is less stable than vitamin A in genuine cod liver oil nearly twice as much being destroyed after exposure to winter sunshine for eleven days [67]

ESTIMATION OF VITAMIN A AND CAROTENE

Vitamin A can be estimated biologically physically by absorption spectra estimations and chemically by colour reactions Since essentially the same methods are used in the estimation of carotene these will not be fully described

The Biological Estimation In biological estimations—which have been fully discussed by Coward [68] in her classical book on the subject—either the curative or the prophylactic method can be used that is the potency of the substance being tested is estimated either by its capacity to cure the symptoms of vitamin A deficiency or by its capacity to prevent their onset Of the two methods the curative is generally considered more satisfactory, though it has been criticized firstly on the grounds that the ill health of the vitamin A depleted animals at the beginning of the estimation varies this variation altering their subsequent response to vitamin A and secondly on the grounds that the depletion period during which a vitamin A deficient diet is given is avoided in prophylactic tests Coward [68] has pointed out that neither criticism is valid because firstly deficient rats show no greater individual variations than do normal rats in their response to vitamin A and secondly in the prophylactic test the animals at the beginning have different amounts of vitamin A already esponse which can only be ignored if r of rats are used Various symptoms of cure of the deficient state Of these change in weight has been the most widely used and has the great advantage that it is easy to measure Against it has been urged that it is not specific for vitamin A—even though vitamin A has a specific action on growth [69]—since any other deficiency has the same effect Xerophthalmia (p 33) and the changes in the desquamated epithelial cells of the vagina (p 36) are sometimes used being only caused by lack of vitamin A Deciding however when early xerophthalmia is present or when it is cured depends on what criteria each worker adopts and so is liable to great variation The changes in the vaginal epithelium are difficult to interpret [70] unless the animals are previously spayed to prevent the changes due to œstrus [71]

Irving and Richards [72] in 1940 found that after seven weeks on a vitamin A deficient diet young rats had a constant degeneration of nervous tracts in the medulla There was only a very small difference between the amounts of vitamin A

degeneration This

Coetzee [73] in 1949

the standard curative method and took two weeks less to perform

carotene By 1934 this sample of carotene was known not to be a single substance and so in its place was put a sample of what was thought to be pure β carotene of which 0 6 microgram was found to be equal in biological activity to 1 microgram of the 1931 standard carotene To avoid confusion the biological value of the International Unit of vitamin A was kept the same so that it was defined as the biological activity of 0 6 microgram of the 1934 standard carotene though this also was later found to be only ninety per cent pure [80]

Now that pure crystalline vitamin A is available the International Unit will be redefined in terms of a given weight of vitamin A or more probably sink into oblivion an International Unit becomes redundant when a vitamin can at last be defined by weight and not only by biological activity

In the U S A crystalline vitamin A acetate dissolved in cottonseed oil is used as a reference standard being known as the United States Pharmacopœia or U S P Vitamin A Reference Standard Units based on this standard are known as U S P units and are equal to International Units [81] The solvent used in assays based on this standard is of the greatest importance (p 12)

In the past U S P units were based on the U S P Reference Cod Liver Oil The vitamin A in this oil was unstable and so the potency of the oil and with it the value of U S P units declined Therefore all work based on the earlier U S P units tends to be inaccurate conversion factors for instance being too high In an attempt to keep the U S P units constant in value a succession of Reference Oils were introduced one after the other but even the third and last by 1949 had lost about twenty five per cent of its activity [81]

Other units such as blue units Moore based on the depth of the blue colour of the Carr Price reaction were used in the early days of vitamin A but they could not be accurately converted into International Units and now have only an historical interest they and their probable values are reviewed by With [82]

PHYSIOLOGY OF CAROTENE OR PROVITAMIN A

Vitamin A is formed in the body from certain of the red or yellow fat soluble plant pigments known as carotenoids No animals can apparently make carotenoids for themselves nor form vitamin A from any other source

Only a few of the carotenoid pigments however have the chemical groupings (p 3) essential for their conversion into vitamin A These are generally collectively referred to as carotene

In calling carotene provitamin A there is the unfortunate implication that as regards nutrition it and vitamin A are the same This is nonsense The vitamin itself is only found in fatty fish or animal foods during digestion it like its accompanying fat is virtually completely absorbed once absorbed it is ready for use by the body Carotene on the other hand has none of these advantages generally locked within the cells of fat free vegetables it has first to be liberated during digestion which often does not occur next it has to be absorbed into the wall of the intestine which again may not occur especially in the absence of fat once within the intestinal wall it has to be converted *there* into vitamin A since if carotene once reaches the blood unconverted it remains unconverted and valueless finally its conversion is largely dependent on age and on the general health of the body It is therefore not surprising that carotene with all these reasons why it may fail to be converted into vitamin A is never the equivalent of the vitamin in practical dietetics

Morton [39] gives an excellent account of the structure of the common and rare provitamin A carotenoids and also of those which have no provitamin activity Of the former only al ta and gamma carotene

and cryptoxanthine are important. All these can exist in many stereoisomeric forms, but the all-*trans* forms are virtually the only ones which occur naturally, the others either being formed artificially during, for instance, absorption on alumina [44] or by plants under artificial conditions, thus the buds of *Mimulus*, or monkey plant, opening in diffuse light in jars of water form *cis* compounds [78]. The biological activity of the carotenoids and their isomers is discussed on p. 15.

Function of Carotene. Plants contain many carotenoid pigments which are valueless to mammals and to ... he subject of this book, but in passing it is ... they are so widely distributed not only ... lticellular organisms, yet their function is still obscure. Goodwin [607] has studied their synthesis in bacteria and fungi. Wald [84] has pointed out that their only universal value in plants seems to be to act as a receptor in photokinetic systems—that is, those concerned with directed movements in response to light. He has enunciated the profoundly significant general theory that "Within the entire range of living organisms, animal and plant, light-sensitive structures regularly, and perhaps universally, contain carotenoids, the class of substances which include the vitamins A. In this respect the appearance of vitamin A_1 in the mammalian retina continues an association which extends so far as is known throughout the eyes of vertebrates and the photosensitive organs of multicellular plants, algæ and fungi."

For mammals and birds carotene itself has no value except as a precursor of vitamin A: the control animals in innumerable experiments on vitamin A requirements, etc., have shown that animals develop and remain healthy on diets containing vitamin A but devoid of carotene, while even on normal diets rich in carotene virtually no carotene is absorbed into the blood by many animals, such as the sheep, goat or rabbit [85], while in other animals, such as the cow and man, the carotene in the blood is only due to fortuitous and valueless permeation through the intestinal wall. The presence of "carotene" in bone marrow, the corpus luteum and suprarenal glands has been held to suggest it has some function of its own, but the only recent work on this subject shows that the pigment in these organs—at least in the goat—is not carotene [85]. Pigments which are able to enter into hens' eggs must contain two or more OH groups [87] and so are not provitamin A carotenoids [86, 87], and the embryonic chick can develop normally with virtually no pigments [87]. Claims that carotene is present in eggs [88] are probably due to the investigators not differentiating between the absorption spectra due to carotene and those due to substances such as lutein and zeaxanthine. With's contention [89] that some carotenoids are themselves vitamins—at least for the chick—is incorrect; some of its fallacies have been pointed out by Hickman [90], while others, such as the different efficiency of conversion of the various provitamin A carotenoids to vitamin A under different dietary conditions (p. 8), will occur to the reader amused by this biochemical red herring.

Absorption of Carotene. In man the absorption of carotene *from normal diets* is extremely poor; so poor that food tables can only give the vaguest guide as to how much vitamin A is indirectly provided by vegetables. As a rough guide it may be said that the carotene in green leafy vegetables is two to three times as well absorbed as that from red or yellow vegetables like carrots [91], but at very best more than half will probably be wasted in the fæces. The whole matter, however, is very confused: one investigation [92], for instance, showed that twenty per cent. of the carotene in raw carrots is absorbed and only five per cent. if the carrots are cooked; a second investigation [91] showed the opposite, absorption being one per cent. for raw carrots and nineteen per cent. for cooked carrots; while a third investigation [93] showed that about twenty-five per cent. is absorbed however carrots are cooked, this amount being doubled if carrots are homogenized

Figures for spinach vary as widely [94, 95], but probably its carotene and that of green peas [95] and cabbage [93] is relatively well absorbed. One vegetarian did not absorb carotene better than men on normal diets [92] There is no evidence that carotene is one of the vitamins synthesized by the bacteria of the bowel [93, 104], though its excretion in the fæces may continue for a week after it has been eaten [96], and its absorption for two or three days [97, 98]

Children and infants absorb or at least convert carotene into vitamin A very inefficiently. *Nicholls and Nimalasuria* [99] report that carotene is not satisfactory for the cure of children suffering from lack of vitamin A, while Van Zeben [100] does not even admit that carotene acts as provitamin A for infants, since he found that only five per cent. of the carotene in cooked spinach was absorbed, and eight per cent. of that in canned tomato juice

The placental barrier [101] is not easily passed by carotene; in new-born infants the level of carotene in the blood is about one-fifth [102] or one-tenth [103] of that in the maternal blood, though there appears to be some definite relationship between the two [103]

Animals differ widely in their capacity to absorb carotene, this is very poor in the cat [104], excellent in the rabbit, sheep and goat [85], while in the rat it is known to depend on so many factors, such as the amount of fat in the diet [104], that ninety per cent. of ingested carotene may be absorbed or ninety per cent excreted [104]

Fat is necessary for the efficient absorption of carotene Wilson [105] reports that in man on a fat-free diet the absorption of carotene is nearly halved but increasing the fat in the diet above thirty per cent does not lead to any further increase in carotene absorption [106] Kreula [107] finds that ninety per cent of the carotene in finely grated carrots is excreted in the fæces when no fat is given, but this falls to fifty per cent if carotene is given dissolved in oil, and falls as low as thirty per cent if such carotene is given not in one dose but in two Similar results have been obtained in rats [104], though colloidal carotene in a glucose solution is well absorbed from fat-free diets [108] Probably the importance of fat is due to its dissolving the carotene and so presenting it for absorption in a fine emulsion

Bile is necessary for the absorption of carotene, since in animals after ligaturing the common bile duct, or short circuiting it into the colon, carotene is not absorbed by mouth unless it is given with bile salts [109], while Irvin and others [110] have shown by using isolated intestinal loops that lipase is as important as bile

Liquid paraffin seriously interferes with the absorption of carotene by dissolving it from the food in the intestine, with the result that it is excreted with the paraffin in the fæces Curtis [111] and his collaborators have shown by careful work on men that on a high carotene diet the carotene level in the blood only rises to half the normal value when 20 ml of paraffin are taken thrice daily after meals; taking it twice daily has nearly as bad an effect, and even taking it once at night has some effect Emulsions of paraffin and agar-agar acted in the same way as paraffin Alexander and others [113] have confirmed these findings for ordinary commercial salad dressings, which may contain over eighty per cent of mineral oil, and similar results have been obtained in work on animals [110, 112] This example of the injurious effects of liquid paraffin will be found to be reduplicated with all the fat-soluble vitamins, but in spite of repeated protests in the medical journals ignorant doctors and rapacious food manufacturers will probably continue to prescribe or sell liquid paraffin overtly or covertly

The solvent in which carotene is given or in which it is dissolved during digestion appears, from the work reviewed above, to be the chief factor determining its absorbability But none of the solvents normally available during digestion are ideal for the absorption of carotene; this is of more

than academic interest because realizing it enabled Koehn [126] in 1948 to perform a completely revolutionary piece of work which showed that given the perfect solvent—*n* hexane—all *trans* beta carotene was as active weight for weight as vitamin A itself This work—which has been confirmed [606]—was revolutionary because until it was published and even much later whenever the biological activity of pure vitamin A was assayed by comparing it to that of the beta carotene of the International Standard (p 9) both were given dissolved in oil with the result that roughly all the vitamin A but only about half the carotene were absorbed Therefore it was erroneously concluded that weight for weight vitamin A was twice as active as carotene when in fact they were both equally active Fortunately methods of assay were so standardized that this fundamental error over the solvents employed occurred in all laboratories so that they all gave congruous results when using carotene in the assay of vitamin A in oils etc

Illness may prevent the absorption of carotene thus diarrhœa [97] fever [97 114] and cœliac disease [114] have been found to increase the fæcal loss of carotene probably because in these conditions fat absorption is impaired Other factors which impair carotene metabolism are considered below when discussing the conversion of carotene to vitamin A

The transfer of carotene across the gut wall probably depends according to Drummond and MacWalter [115] on the formation with bile acids of a water soluble diffusible complex though it would seem from Frazer s work [116] that it might also be absorbed dissolved in any fat which was so finely emulsified that it did not require lipolysis for its own absorption Having passed into the lacteals from the intestine [117] the carotene is transported in the blood as a protein compound the protein probably being albumin [119]

Storage and Excretion of Carotene Many animals (p 14) never absorb carotene itself into the blood while others absorb and store very large amounts—chiefly in the liver though the body fat may also be impregnated with this pigment we have been told for instance that at operation the fat of prisoners of war in the Far East who had to eat huge amounts of red palm oil was a startling scarlet The paper by Jensen and With [120] should be read for a very interesting account of carotene storage in men mammals birds and reptiles there may be the widest variations even between closely related species living on similar diets

After injecting carotene into the tissues it may remain unabsorbed [121] or if directly injected into the circulation it is treated as a foreign body and is removed from the circulation by the reticulo-endothelial system [58 118]

The fate of carotene after it has been injected or has been absorbed from the gut but not converted into vitamin A during this absorption is obscure A very small amount may perhaps be re excreted into the gut [122] some may be excreted by the skin though not the kidneys when levels in the blood are abnormally high (p 78) but when very large amounts are consumed the greater part at least in the rat is destroyed by the body [123]

Conversion of Carotene to Vitamin A One molecule of all *trans* beta carotene is converted into two molecules of vitamin A this conversion taking place in the wall of the intestine

The above is what happens when carotene is fed to an animal under ideal conditions it is not what happens under ordinary conditions for either man or laboratory animals The subject is most simply reviewed by considering firstly what chemical changes occur in the carotene molecule during conversion secondly what is the site of this conversion in the body thirdly what factors hinder or facilitate this conversion

The chemical change which converts beta carotene to vitamin A is probably oxidative cleavage of the molecule (p 3) at the middle of the central aliphatic chain two identical molecules of vitamin A aldehyde being

formed which are then reduced to vitamin A alcohol, that is, to vitamin A itself. In support of this theory is the work of Hunter [37], who succeeded in making small amounts of vitamin A by the oxidation of carotene, and of Glover and his colleagues [124], who have shown that vitamin A aldehyde is converted to the alcohol in the gut wall. Koehn [126], however, holds that the beta carotene molecule is not oxidized but undergoes hydrolytic fission. The changes which occur in the other provitamin A carotenoids are considered later when discussing the factors which affect conversion.

The site of conversion of carotene to vitamin A is the wall of the gut. That this is so in the rat was first shown by Glover, Goodwin and Morton in 1947, and it has been thoroughly confirmed by their own and their collaborators' subsequent work on the rat [127], and on the sheep, goat and rabbit [85], and by the work of Thompson, Ganguly, Coates and Kon on the rat and pig [128, 132] and chick [132], and of Americans, again on the rat [122, 129]. All this research has proved that when carotene is fed by mouth (*a*) it is not converted to vitamin A within the lumen of the gut; (*b*) it causes storage of vitamin A but not carotene in the liver; (*c*) vitamin A appears within the wall of the gut in large amounts before it appears in the liver; (*d*) vitamin A disappears from within the wall of the gut as it increases in the liver; (*e*) no carotene appears in the lymph from the thoracic duct or in the portal or systemic blood *but* vitamin A appears in the lymph. Further, the injection of carotene into the circulation or tissues causes no hepatic stores of vitamin A, and animals so injected die from lack of vitamin A when there still remains in their livers enough of the injected carotene to keep them alive for many months were it converted to vitamin A [121, 122]. No explanation of all these results is possible except that carotene is converted to vitamin A only within the wall of the gut.

However, there are some observations that at first sight do not appear to be congruous with those just reviewed. Until 1947 it was believed that the liver—by virtue of a hypothetical enzyme called carotinase—was the site of the conversion of carotene, this belief resting partly on experiments in which vitamin A was said to be formed after carotene was perfused through the liver or liver tissue was incubated with carotene, and partly on experiments in which carotene when rubbed or injected into animals showed vitamin A activity. The perfusion or incubation experiments have been reviewed by Glover and others [127]; they were contradictory and the yields of vitamin A so small that they were within the limits of experimental error. But the experiments based on the inunction or injection of carotene cannot be so easily ignored; thus Eddy [130] showed in a very careful experiment that carotene solutions when rubbed for four minutes into the shaven skin of rats—the skin being thereafter carefully washed—promoted growth, even though about three times as much carotene was required as if it were taken by mouth. Lease [121] and later Sexton and others [122], showed quite definitely that injected carotene could cure ophthalmia and restore growth in vitamin A deficient rats for a short period of time. But the animals of both these investigators ultimately died of lack of vitamin A when there was enough carotene in their livers to have kept them alive for many months had this been convertible to vitamin A. The probable explanation of this definite but transitory effect of carotene when introduced directly into the body is that, while most remains at the site of the injection or is captured and destroyed by the Kupffer cells of the liver, yet a little, while still circulating in the blood, is excreted into the gut and is then reabsorbed and converted by the gut wall to vitamin A. In support of this is the recovery from the fæces of 5.5 per cent of injected carotene [122]. Further, vitamin A depleted animals, whether they are injected with one large or one relatively small dose of carotene, die, after a brief recovery, within the same period [121] though repeated injections enable animals to grow normally [131], which suggests that it is only during the short time when carotene is circulating in the blood,

and so being excreted in the intestine, that it is available to form vitamin A In other words, the injection or injection of carotene is merely a most wasteful method of introducing carotene into the gut

The factors affecting the conversion of the provitamin A carotenoids to vitamin A are firstly the stability of the carotenoids within the lumen of the gut, secondly their absorbability (p 11), and thirdly their convertibility after absorption

The stability of the carotenoids within the lumen of the gut largely depends on vitamin E protecting them against oxidative destruction by rancid fat [133], but the matter is complicated by vitamin E also protecting vitamin A within the body, so some of the apparent protection of the carotenoids—as judged by their growth promoting power—may really be due to vitamin A not being destroyed after its formation. The subject is discussed fully in relation to vitamin A on p 23, but here should be mentioned the work of Quackenbush and his collaborators [134] on linoleic acid, which is one of the essential unsaturated fatty acids (p 671), they report that carotene dissolved in ethyl linoleate is unstable *in vitro* and is ineffective in curing vitamin A deficient rats unless protected against oxidation by vitamin E or hydroquinone This is contradicted by Sherman [135], who states that carotene is stable in ethyl linoleate even when both are incubated with the contents of the rat's stomach, and ethyl linoleate does not decrease the amount of carotene in the fæces Sherman [136] therefore believes that the inhibitory action which the esters of linoleic and linolenic acids have on the metabolism of carotene occurs after absorption even though he reports that this inhibitory action does not occur if the esters are fed six hours after the carotene [135] or vitamin E is given [136] On the balance of evidence it would appear that the unsaturated fatty acids only disturb the metabolism of carotene by oxidizing it before it can be absorbed

The convertibility of the carotenoids—apart from that of all *trans* beta-carotene, which has been discussed on p 13—is still unknown From their chemical structure (p 4) it is obvious that all isomers of beta-carotene could be converted into an equal weight of vitamin A, while the other carotenoids could form half this But from the reported biological activities of the carotenoids and of their stereoisomeric forms it would appear that, even when allowances are made for differences in absorbability [137], some carotenoids do not form as much vitamin A as their structure suggests is possible In other words, they are not easily converted, a large proportion being destroyed before or after absorption or, possibly, some part of the carotenoid molecule which does not form vitamin A—such as the beta-ionone nucleus—may destroy vitamin A after its formation and so give a false picture of how much was originally made [137] Zechmeister [78] in 1949 discussed some of the particular problems raised by the stereoisomers and suggested that their different biological activities are due to whether or no they are the right shape to fit into the enzyme system responsible for their conversion Deuel and his co workers [138] found, judging by the gain of weight in rats and ignoring differences in absorption, the following figures of relative activity β carotene 100, neo β carotene B 53, neo β carotene U 38, α carotene 53, neo α carotene B 16, neo α-carotene U 13, γ-carotene 28, pro γ-carotene 44, cryptoxanthine 58, neocryptoxanthine 42 Johnson and others [139] have largely confirmed these findings both for the rat and for the chick studying respectively storage, and growth and storage That α carotene has roughly half the activity of β carotene judged by its effect on growth [138] and only one quarter judged by hepatic storage of vitamin A [137] is proba

laboratories at

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With (p 11) la

their own right on his erroneous claim that cryptoxanthine had double the activity of β carotene

Many other factors at least in animals affect the value of carotene as a source of vitamin A Thus myxœdema (p 45) hepatic disease [101] phosphorus poisoning [141] some anæsthetics or drugs [128] and impaired bowel motility [128] may all have an adverse effect as does lack of vitamin E (p 23) and possibly of choline (p 24) and also the consumption of soya beans [142] Lutein or xanthophyll when fed in large amounts—with ample vitamin E—at the same time as carotene [137 143] or vitamin A [143] reduces the amounts of vitamin A laid down in the liver [137 143] though it does not hasten the depletion of preformed stores [143] Since lutein does not increase the excretion of carotene [137] it presumably accelerates the destruction of carotene and vitamin A within the wall of the gut or by virtue of its alpha ionone ring within the lumen of the gut [137] The contrary evidence that lutein in the absence of vitamin E protects carotene from destruction [144] is probably incorrect [126]

Utilization of Carotene Moore [145] found that in animals small amounts of carotene added to diets deficient in vitamin A were utilized as well as vitamin A but that increasing the carotene decreased the percentage stored till at very high intakes only about one to two per cent was converted into vitamin A five to ten per cent was lost in the fæces the remainder apparently being destroyed in the body [123] He also states that it is impossible to cause the symptoms of over dosage with vitamin A by giving carotene Gray [146] reported that in rats at levels of intake above the minimum to prevent symptoms of a deficiency vitamin A was six to ten times better utilized than carotene while Guilbert and others [147] and English workers [93 148] have also emphasized that at optimum levels of intake 1 I U of vitamin A is equivalent to about 3 I U of carotene Children utilize carotene very badly (p 12) From all this it appears to be certain that carotene is a far less satisfactory source of vitamin A than is vitamin A itself (see also p 50)

PHYSIOLOGY OF VITAMIN A

Sources Herbivorous animals depend entirely on carotene for their vitamin A Omnivorous animals like man obtain vitamin A partly from carotene partly from animal foods in which the vitamin itself is present Some purely carnivorous animals like cats are almost unable under normal conditions to convert carotene to vitamin A [104] How fish acquire such large stores of vitamin A in their livers or of what use it is when stored is obscure though in some fish it may possibly be necessary for the transference of fat across the gut wall [149] though this does not appear to be so in animals [150 151] The seaweeds and plankton which form the basis of marine food contain carotene but the small fish and crustacea which act as it were as intermediaries between the plankton and the larger fish do not contain vitamin A or carotene though they do contain carotenoid pigments The fish may use these as precursors of vitamin A carefully storing it up so that it increases in amount in the liver with increasing age [152] or they may themselves apparently synthesize the vitamin [153] also converting carotene should it be available [154] The crustacea however on which Antarctic whales feed appear to contain vitamin A itself and so may be the source of the large hepatic stores of the vitamin in these mammals [128 602]

Absorption Vitamin A only occurs naturally as the ester therefore fat and vitamin A from the point of view of absorption behave in the same way both being in essence esters of fatty acids and alcohols Hence any conditions which impair the absorption of fat equally impair the absorption of the vitamin Further Fraser [116] has shown that while fat must normally be hydrolysed before absorption yet it is possible for it to be

absorbed chemically unchanged if it is very finely emulsified the same is true of vitamin A

Vitamin A undergoes no change during intestinal absorption since it is active when placed in the eyes [189] or when given by inunction through the skin [130] and is as effective when injected (p 27) as when given orally, though for injection the solvent is most important propylene glycol but not cod liver oil being satisfactory [190]

The transfer of vitamin A across the gut wall is in the form of the alcohol the naturally occurring vitamin A esters being first hydrolysed like the other esters of the fatty acids by the enzymes of the gut the evidence for this is discussed below under *Lipolytic enzymes* Before passing into the lacteals [132] the alcohol is again combined with fatty acids since in the chyle from a patient with a chylous fistula Drummond and his collaborators [117] found the vitamin in the form of an ester this has been fully confirmed for animals [85 128 132 156] the absorption taking place in the upper or middle part of the small intestine [156 164] As all the vitamin A esters found in the rat s liver appear to be very similar as regards their fatty acids it is probable that there is a selective utilization of certain fatty acids for esterifying the vitamin [146] In the blood vitamin A is present not only as the ester but also as the alcohol (p 24) probably forming a compound with albumin [119]

Factors Influencing Absorption Before discussing these it must be emphasized that in all the clinical work quoted below—unless otherwise stated—the efficiency of the absorption of vitamin A has been judged by how great a rise has occurred in the level of the vitamin in the blood (p 25) after it has been given by mouth But such a rise merely shows that absorption has occurred and has temporarily outpaced storage it does not show how great the total absorption has been For instance in normal adults the rise following the consumption of an aqueous dispersion of the vitamin is two to three times as great as that following the consumption of the same amount of the vitamin dissolved in oil but the total absorption in both cases is the same all the vitamin being absorbed Not realizing how fallacious are rises in blood levels as guides to absorption has caused much confusion claims repeatedly being made for instance that dispersing agents improve absorption when all that can be justifiably stated is that such agents hurry absorption—for good or more probably for ill (p 18)

The ester and the alcohol—and the acetate [192] for all practical purposes—are equally well absorbed by man [160 163 176] except in certain diseases (p 17) Both are also equally well absorbed by the cow [179] though the laying hen [180] is reported to utilize the ester far better at high levels of intake while certain solvents on the other hand hinder the hydrolysis and so the absorption of the natural esters by laboratory animals and chicks [178] The solvent indeed is very important when assaying vitamin A since for instance some solvents make the natural ester appear to be only half as biologically active as the acetate or alcohol [178] To avoid this confusion all substances before being assayed and also the standard of reference should be saponified

Lipolytic enzymes appear to be necessary for the absorption of vitamin A since it seems probable from work on rats [128 155] and bovines and sheep [156] that the naturally occurring vitamin A esters if unemulsified have to be hydrolysed to the alcohol—like other esters of fatty acids—before they can be absorbed This is supported by the poor or slow absorption of the ester compared to the alcohol by children who lack lipolytic enzymes owing to cystic fibrosis of the pancreas [157 158 159] and the improved or more rapid absorption of the ester by such children when pancreatic enzymes are given [158 159]

Bile assists the absorption of vitamin A since adults [160] and children with jaundice have a poor or slow absorption which is improved at least in

the latter, when bile salts are given [166, 167] and children dying of congenital atresia of the bile ducts have been reported to show signs of a deficiency of vitamin A [168] which was probably not due to the jaundice, since jaundiced animals utilize vitamin A [109] In animals, however, ligaturing the common bile duct or anastomosing it with the colon does not interfere with absorption [109]

Decreased intestinal motility probably impairs absorption of vitamin A—as it does of carotene (p 16)—since neostigmine and cascara increase or hasten absorption in fibrocystic disease of the pancreas [169], while in normal subjects atropine delays or decreases it [170]

Liquid paraffin hinders the absorption of vitamin A in the same way as it hinders the absorption of all the fat soluble vitamins Thus *Anderson* [171] found that when liquid paraffin was given with large doses of vitamin A about a quarter of the latter was excreted with the fæces, being dissolved in the paraffin, one patient, for instance, when given 20,000 I U by mouth normally only excreted 800 I U, but this amount rose to 5,000 I U when liquid paraffin was taken The level of vitamin A in the blood may not be depressed even by large doses of paraffin taken for many weeks [113] but this is no argument in favour of this harmful aperient [172] since the blood levels may remain normal after months on a vitamin A free diet [93] if there are ample hepatic stores on which to draw

Emulsifying agents apart from bile, which has been discussed above, have been known for some years to hasten and sometimes to improve absorption of vitamin A Thus Aldersberg and Sobotka [173] in 1943 showed that *when vitamin A was given with 10 grams of impure lecithin the subsequent* rise in serum vitamin A was roughly double that which occurred when no lecithin was given Vitamin A as it occurs naturally emulsified in milk is almost perfectly absorbed [163], and the same is probably true of vitamin D (p 537) It is most improbable that natural, common and physiologically normal emulsifying agents could be harmful, but it is far otherwise with the far more efficient artificial agents such as sorbitan monolaurate polyoxyethylene sorbitan monolaurate and polyoxyethylene monolaurate which were first used about 1947 Claimed by the makers to be safe and harmless it [illegible] workers [175] [illegible] were highly [illegible] the intestinal tract It is true that these compounds [illegible] er cent of the *animals' food*, while *in children* they are never given in any but relatively minute doses, but even so it is difficult to believe that a substance which kills animals in a few weeks is a desirable addition to vitamin preparations which are often taken by infants and children for many years Further, an emulsifying agent which, when used to make an "aqueous dispersion of vitamin A, is so efficient that it quintuples the normal rise in serum vitamin A which follows the giving of ordinary halibut liver oil [160] cannot but open the door to the entry into the body of a multitude of substances—such as liquid paraffin—which in normal circumstances are excluded Again the abrupt flooding of the body with *vitamin A may overwhelm or pervert* storage [181] All this of course does not mean that emulsifying agents are *not of value, when given* under careful medical supervision for the treatment of children and adults who cannot absorb fat soluble vitamins in a normal manner But it would seem that vitamin preparations sold to the public should never contain emulsifying agents, and warnings have already been given that such preparations—apart from other dangers—enhance the risk of vitamin A poisoning [163]

Emulsifying agents such as those mentioned above and also possibly dextrimaltose [160] when used to turn oils containing vitamin A into aqueous dispersions enormously hasten and may increase absorption both by man at all ages and by animals [160, 161, 163, 164] and chicks [165] Thus in

animals about three times as much vitamin A may be absorbed [164] and stored [161] from aqueous dispersions as from oils, absorption from the former being quicker and taking place higher in the small intestine [164]. In
I.U. of vitamin A
U., while the same
. 4 I.U.; the comparable figures for normal infants are 200 and 1,000; for children given 5,000 I.U. per pound of body weight, 600 and 4,000; and for adults given 500,000 I.U., 1,750 and 4,500 (Figs. 6, 7). The normal infants excreted in their fæces an average of thirty-eight per cent. of the vitamin A in the oil, and seven per cent. of that in the aqueous dispersion; the percentage excreted was the same whether 12,500 or 35,000 I.U. was given, and the absorption from the oil was not improved when it was diluted from 60,000 to 10,000 I.U. per ml.

The amount of the emulsifying agent is important; thus in children 6 ml of an aqueous dispersion containing roughly 50,000 I.U. and eleven per cent. of *sorbitan monolaurate polyoxyalkylene* raises the serum vitamin A to 400 I.U, while if the emulsifying agent is increased to twenty per cent. the serum level is raised to 13,000 I.U. [176]

Findings congruous with the above as regards the hastening or enhancing of vitamin A absorption by aqueous dispersion have been reported by many workers for adults in health [160, 164, 176] and with most types of illness [177], for children [158, 160, 162, 163, 176] and for infants [160, 176] Children with fibrocystic disease of the pancreas [158, 160, 162, 163] and cœliac disease [158] and adults with obstructive jaundice [160] absorb vitamin A from an aqueous dispersion—judged by serum levels—better than or as well as normal patients absorb vitamin A from oil (Figs 6, 7)

Age has an important effect on absorption, which is virtually complete in adults [96] but poor in infants, who, judging by fæcal excretion, only absorb about two-thirds of a dose of 35,000 I.U given in oil [160, 163].

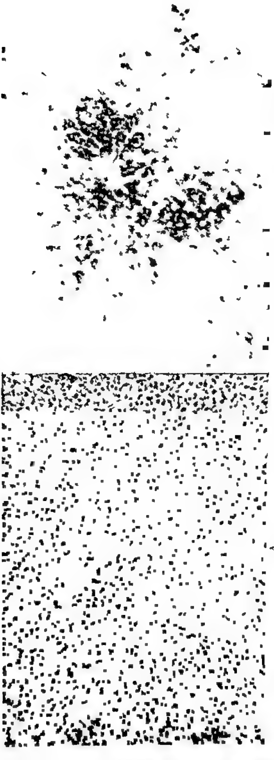
The placental transfer of vitamin A to the fœtus appears to be controlled by various factors which have not been fully investigated. Byrn and Eastman [102] found the average level of vitamin A in the blood of fifty newborn infants was 91 3 I.U. and that of the mothers 106 3 I U., but there was no constant relationship and wide differences occurred; this has been confirmed in 143 mothers and children by Lund and Kimble [103], who also noted that the fœtal blood vitamin A might be higher than the mother's when this was low, and further that the levels in a pair of identical twins were 30 and 71 I U. per 100 ml. Human fœtuses and infants tend to have low stores of vitamin A (p. 22)

In animals [188, 193, 194, 195] fœtal stores depend on the richness of the maternal diet in vitamin A and, to a slight extent, fat [193]. The fœtal rat can only store about 5 to 10 I.U however much the mother has consumed [195], but the fœtal calf does not appear to be so limited in its storage capacity [188], while the hen can transfer to the egg very large amounts [86, 180], especially when given vitamin A as the ester [180]. Vitamin E does not increase placental transfer to the kid or piglet, but possibly does to the lamb [194]

Diseases of many different types are often stated to decrease or delay absorption. But the evidence for this is almost wholly based either on the amounts of vitamin A found *post mortem* stored in the liver or on the levels of vitamin A in the blood during life. Therefore the reader should consult
and on blood levels as well as the sections
r information as to how disease may affect

A is confined to the liver for all practical
iding in his early work that the liver of rats
ed 100,000 times the amount of vitamin A

present in the storage fat of the body, while no other organs contained any Later work (p 48), however, showed that with *moderate* stores in the liver small amounts of vitamin A were always present in the kidneys and sometimes in the lungs When the diet was very rich in vitamin A, so that the liver stores were five per cent of its dry weight and could in theory have lasted the rat for 200 years, the *lungs* stored more than the kidneys, the amount being larger in both organs than those found in normal livers. The suprarenal glands also, but inconstantly, stored vitamin A in large amounts, while all the other tissues of the body contained traces In the *pig* [128] also the liver is virtually the only store house for vitamin A, the walls of the stomach and small intestine, the intestinal lymphatics and lymph nodes, the pancreas and gall containing only traces, and the kidneys very small amounts In man the adrenals, testes and lactating breast are all said to store vitamin A [58], as well as the *ovaries from infancy* to the climacteric [182] Moore and others [183] have shown that it is the healthy human kidney which stores vitamin A in nephritis and respiratory diseases the kidney generally contains none Popper [58], however, states that it is only the diseased kidney which contains vitamin A, though this is not congruous with the distribution in healthy *animals (see also p 48)* Tumours only store vitamin A if it is present in the parent cells, and the retina if it is light adapted [58]



A B

FIG 3 Photomicrographs of a biopsy specimen from the liver of a patient with peptic ulcer without damage to the liver A, routine histologic examination normal structure of the hepatic parenchyma B examination with fluorescence microscope vitamin A fluorescence imparted by fine lipid droplets at the edge of the liver cells and in the Kupffer cells (*See also Figs 4 and 5*)

The level of vitamin A in the blood and the cerebrospinal fluid is discussed on p 24

The average storage in human adults from the ages of fifteen to fifty nine was given by Moore [184] between 1931 and 1935 as being 220 I U per gram of wet liver, but between 1941 and 1944 this storage had increased to 324 I U [93], though the figures are not strictly comparable since the specimens

in the two surveys came from different areas in England. For children the respective figures were 130 and 550 I.U., while for the elderly they were 158 and 300 I.U. In Scotland [185] in 1945 the average figure between the ages of twelve and eighty was 504 I U. It is a moot point whether an increase in storage means better nutrition or worse; whether it means more dairy produce or more vegetables eaten to stave off hunger. The differences between individuals is remarkable, figures range from 23 to 2,400 I U in adults dying in accidents [93], while in infants and young children dying at birth [186] or later from various causes [187] figures have varied from 14 to 134 I U In the five months' embryo the stores of vitamin A have been reported to be high [58] Braun and Carle [188] in 1943 reviewed the literature on fœtal and infantile stores in man and animals

Both the true hepatic cells and the Kupffer cells store vitamin A [58, 196], so that in phosphorus poisoning of the hepatic cells good stores remain [118, 184], while "blocking" the Kupffer cells reduces storage [197] Popper [58] states that his fluorescence microscopy shows that the Kupffer cells (Figs 3 and 4) are the last cells to give up their stores of vitamin A and the first cells to replenish them but Glover and Morton [191], from a review of the literature, believe that the evidence points to the Kupffer cells only storing vitamin A after the normal storage space in the true hepatic cells has been filled They also suggest that, while the true hepatic cells contain a lipolytic enzyme which can hydrolyse the stored vitamin A ester and so release it as it is needed into the blood as the active alcohol (p 24), the *Kupffer cells contain no such enzyme* and so can only slowly

B

A

FIG 4 Photomicrographs of a biopsy specimen from the liver of a patient with disease of the gallbladder with jaundice of short duration and without clinical signs of damage to the liver A, routine histologic examination extensive lymphocytic infiltration in the enlarged periportal field No visible damage to the parenchyma B, examination with fluorescence microscope irregular vitamin A fluorescence is imparted almost only by Kupffer cells. The bile casts in the enlarged bile capillaries appear black (*See also* Figs 3 and 5.)

release the inactive ester In other words, storage in the Kupffer cells is physiologically valueless, playing no part in keeping vitamin A alcohol at a constant level in the blood

Factors, apart from Intake, affecting Storage *Disease* Moore [184] gives the following figures, in International Units of vitamin A per gram of wet liver, for adults dying of various diseases thyrotoxicosis 310, diabetes 300, poisoning 170, hypertension 120, conditions of the gall bladder 110, gastric ulcer 110, coronary thrombosis 110 tuberculosis 96, syphilis 95, endocarditis 90, bronchiectasis and bronchitis 80, subacute nephritis 75, peritonitis 75, enteritis and colitis 74, meningitis 73, pneumonia 63, empyema 60, valvular disease of the heart 60, septic diseases 51, prostate 40, chronic nephritis 25, kidney and bladder infections 19 From Scotland [185] low stores have been reported in renal disease, congestive heart failure, chronic infections and, especially, diseases of the alimentary tract In syphilitic [184] and other forms of cirrhosis [198 199] storage is low, and generally [58, 184, 185 198] but not always [59 200, 201, 202] in "hepatic diseases" (see Figs 4 and 5) Fox [203] reports that large doses of vitamin A given to patients with pneumonia who subsequently died, did not increase their hepatic stores

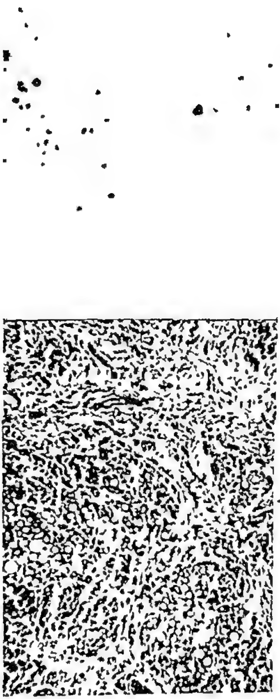


Fig 5 Photomicrographs of a biopsy specimen from the liver of a patient with cirrhosis with jaundice A routine histologic examination extensive proliferation of connective tissue with lymphocytic infiltration and proliferation in the bile ducts Many fat droplets of various sizes in the hepatic parenchyma The cytoplasm of the hepatic cells is dark B examination with fluorescence microscope vitamin A fluorescence imparted by a few irregularly shaped Kupffer cells and by the large fat droplets the fluorescence of which is very low (*See also* Figs 3 and 4)

In children under the age of fifteen [189] the values were tuberculosis 140 measles 110, pneumonia 78 meningitis 68 septic diseases 47 heart disease 15 Some of the children who died from measles had been given large amounts of vitamin A, but by comparison with other children not given vitamin A it appears that they only had stored about seven per cent a figure comparable to the storage of only ten per cent reported by Woo and Chu [204]

A disease may affect storage by decreasing absorption increasing utilization hindering the conversion of carotene to vitamin A impairing the storage capacity of the liver or increasing excretion by the kidney All or any of these factors have been the cause of the figures given above but it may be tentatively suggested (1) that all the diseases where low storage occurs would tend to decrease absorption by a poor appetite fever (p 30) and an unhealthy gut (2) that the low figures found in heart disease are partly due to anoxæmia interfering with conversion and partly to the engorgement of the liver with blood which decreases the proportion of vitamin A to liver weight, (3) that the yellow liver found in patients dying from the uræmia of chronic nephritis (p 47) is due to the presence of carotene which presumably could not be converted to vitamin A because of the toxic condition of the gut (4) that though many of the pulmonary infections with a low storage are similar to those from which vitamin A deficient animals die yet the low storage is not the cause of the infections but the result of poor absorption or poor conversion and increased renal secretion (p 47) all of which might then tend to set up a vicious circle by decreasing the power of resistance of the lungs (p 38)

Vitamin E The sparing effect which vitamin E has on the metabolism of vitamin A may have more than academic importance *since when human diets contain insufficient vitamin A or carotene an increase in the consumption of vitamin E would decrease this insufficiency* [205]

Further when vitamin A or carotene are being assayed biologically equal amounts of vitamin E must be included in the diets of the experimental and control animals as the response to vitamin A will be affected by the amount of vitamin E in the diets [205]

Davies and Moore [206] first suggested that the reason why vitamin E spares hepatic stores of vitamin A [207 208] is that it protects them against oxidation within the liver This idea has been amplified by Hickman and his collaborators [205 209] who believe that vitamin A in vitamin E deficient animals is drained away from the liver to replenish the blood vitamin A which is lost through oxidation within the blood vessels of the gut This oxidation is due to oxidants from within the lumen of the gut diffusing into the blood vessels normally such oxidants are destroyed within the gut by the vitamin E of the food which thus also preserves vitamin A and carotene from oxidation before absorption (Compare the destruction of vitamin E by rancid fat p 615)

The original work which drew attention to the relationship between vitamins A and E was done by Moore [206 207] and by Bacharach [208] The former found that the storage of vitamin A—given weekly as halibut liver oil—in vitamin E deficient rats was increased from two to ten times when the deficiency of vitamin E was removed by giving large amounts of wheat germ oil daily or synthetic vitamin E alcohol weekly Vitamin A storage from carotene was not so greatly affected Bacharach [208] keeping rats for shorter periods than Moore on a vitamin E deficient diet found storage could be increased from twenty five to thirty three per cent by giving large amounts of synthetic vitamin E ester daily physiologically adequate amounts of the ester had no effect

In both the above experiments the intake of vitamin A was high Hickman and his collaborators [205 209] investigated the subject further by feeding vitamins A and E in small and varying amounts to rats Their extensive work should be read in full their chief conclusions being vitamin E increases the growth promoting power of vitamin A and carotene and the survival and depletion times of vitamin A deficient rats there is an optimum ratio between vitamins A and E in the diet which was also noticed by Moore [207] vitamin E is most effective when fed together with vitamin A injections of vitamin E are ineffective the effect of injected vitamin A is enhanced by oral vitamin E a mixture of the three naturally occurring

tocopherols is slightly more effective than α tocopherol alone and much more effective than tocopherol esters, the action of vitamin E is enhanced by reducing agents such as ascorbic acid. In man vitamin E does not affect the vitamin A tolerance curve (p 25) or excretion in the milk (p 52), though it may raise the level in cows' milk [608]. Further relationships between vitamins A and E are discussed on pp 15 and 36.

Choline Popper [58] reports that in rats on a diet deficient in choline with ample carotene no vitamin A is stored in the hepatic or Kupffer cells, though it is present in abnormally large amounts in the kidney. When vitamin A itself is given in the diet the Kupffer cells but not the hepatic cells contain vitamin A. Other workers [210] have thought that a deficiency of choline may hasten the depletion of hepatic stores of vitamin A. The relationship of vitamin A to the other vitamins is described on p 49.

Other Dietetic Factors Dann and Moore [211] observed that in rats extreme wasting from lack of aneurine had no effect on the storage of vitamin A, and beriberi in man does not cause night blindness [214]. Moore [184] found that in man there was no relationship between the stores of vitamin A and the general nutrition. Storage in animals is increased by a high fat diet [212] and not affected by a low protein diet [207]. In rats [210] and chickens [213] a deficiency of vitamin K has no effect on storage. Carcinogens may or may not cause depletion of vitamin A in the liver [215], and depletion is not hastened by 4 hydroxycoumarin, carvone, dimethylaminoazobenzene or flushing out hepatic fat [210]. The factors influencing absorption, and so storage, have been discussed on p 17. Atebrine given to rats impairs both storage and absorption [612].

Blood Levels The ester and the alcohol of vitamin A are both present in the blood, the former according to Hoch and Hoch [216] forming ten to seventeen per cent of the fasting total in both men and women, while Popper [177] gives the figure as twenty per cent. The alcohol appears to be the active form of vitamin A, the ester merely being used for transport from the gut to the liver (p 17) and for storage in the liver. The arguments in favour of this are firstly that Glover and his co workers [217] have shown that in the rat the plasma vitamin A is proportional to the amount of vitamin A alcohol in the liver but is not proportional to the total stores which are chiefly in the form of the ester. Secondly, whenever a large dose of any form of vitamin A is fed to man [159, 177, 192, 216] the amount of the alcohol in the serum remains almost constant, whatever slight rise there may be occurring later than the very large rise in the ester [216]. Thirdly it would seem probable that there would be some protection for the body against the enormous amounts of vitamin A which for a short time after absorption may circulate in the blood, this could be achieved by vitamin A being transported to its storage in an inactive form, such as the ester is thought to be. The factors which influence the levels of the alcohol and the ester are discussed later when considering the factors which influence the total levels of vitamin A.

The average fasting level of the total vitamin A in the plasma of men is about 130 I U or 39 micrograms, and of women about 110 I U, or 33 micrograms, per 100 ml. These are the figures given in 1949 by Moore and Leitner [93], being based on their own work in England and on the reports of other surveys in England and the U S A. Later surveys in the U S A give slightly higher figures, thus the average for 126 men between the ages of forty and ninety was 150 I U, age making no difference (218), for 18 men and 7 women 162 and 120 I U [192], for 100 men and women, 150 I U [219].

Children under sixteen years of age are said to have slightly lower levels than adults in the same family [220], which is supported by the average level of 394 school children in the U S A being 114 I U [221]. In Brussels [93] the average for 41 boys and girls aged about fourteen years was 134 I U,

for 60 boys in Dundee [93] aged about fifteen it was 88 I U, for 11 youths aged about seventeen in the south of England [93] it was 137 I U while in Italy [222] values for infants and children have been reported as being between 52 and 210 I U the lower levels occurring most frequently in infancy Premature infants a few weeks old have been found in the U S A to have values ranging from 52 to 164 I U [160] At birth the average level of vitamin A in one series [103] of 143 infants in the U S A has been reported as 49 I U in another series [102] of 50 infants as 91 I U and in a third series [186] of 108 infants as 76 U S P units which fell to 37 at the end of the second day and then rose again to 61 on the fourth day The factors which affect the levels in adults and infants are discussed later

The individual s fasting blood level may vary so greatly from the average that though it tends to remain a personal characteristic—as is discussed on p 28—it has none of the clinical value of for instance a fasting blood sugar level Thus for apparently healthy adults values have been reported of 30 to 264 I U [218] 66 to 231 I U [192] and under 40 to over 280 I U [93] while for children values have been from 52 to 210 I U [222] and for infants 24 I U [103] to over 160 I U [102] Therefore all that can be deduced from fasting serum levels is that when these are above 300 I U there has probably been within the last few days a very large or toxic consumption of vitamin A (p 81) while readings below 70 I U should suggest the possibility in an apparently healthy man of a genuine deficiency of vitamin A since Lindquist [223]—though his figures may be too high [93]—and Cowell [224] and Pett and La Page [225] have all reported that dark adaptation (p 60) is impaired when levels are as low as 70 I U while the Medical Research Council report [93] states that the three subjects whose dark adaptation had deteriorated significantly had values below 50 I U

Vitamin A absorption or tolerance curves are similar to blood sugar curves showing how the level of the vitamin in the serum alters after an oral dose To construct such a curve the patient who has fasted all night and has had no large dose of the vitamin in the preceding twenty four hours has his fasting level measured by any of the methods which have been fully described [93] An oral dose of the vitamin is then given and serum levels are measured hourly for the first six to eight hours and sometimes over longer periods There is no standardized oral dose for adults the doses have been 50 000 I U [220] or 75 000 I U [177] or 100 000 I U [218] or 134 000 I U [192] or 250 000 I U [220] or 500 000 I U [160] For children the doses have been 25 000 to 50 000 I U [221] or 5 000 I U [160 222] to 6 000 I U [162 226] per pound of body weight and for infants 35 000 I U [160] irrespective of weight The maximum rise when the vitamin is given dissolved in oil occurs within three to six hours after which the level falls rapidly till about the eighth hour and then very gradually not returning to normal until about the end of twenty four hours The larger the dose the higher the level reached though the shape of the curve is not altered [221] When the vitamin is given as an aqueous dispersion—a subject very fully discussed on p 18—the rise is far greater and the maximum level is attained sooner Figs 6 and 7 show the kinds of curves which are obtained and how they differ with sex the size of the dose etc

But while it is possible to draw an average curve this curve may differ very considerably in its shape and in [illegible] from that obtained from a normal healthy [illegible] for instance given by Lewis and others [16[illegible] show this very clearly or again Erhr[illegible] maximum levels attained in adults all given the same dose was between 133 and 1 234 I U

In disease however the value of tolerance curves is not vitiated by their normal lack of uniformity since they are only used clinically as an aid in confirming—in the unlikely event of such confirmation ever being needed

—a diagnosis of mongolism [227], lambliasis or giardiasis [229], sprue [173], cœliac disease [158, 159 162], congenital atresia of the bile ducts [168, 228], intestinal tuberculosis [159] and cystic fibrosis of the pancreas [158, 159, 160, 163], in all these conditions the tolerance curve (Fig 7) with the vitamin dissolved in oil differs far more from the average than does the curve of any normal individual The level of vitamin A *rises very slowly to a relatively very low maximum in about nine hours and then falls so gradually that at the end of twenty-four hours it may still be four times as high as it was at*

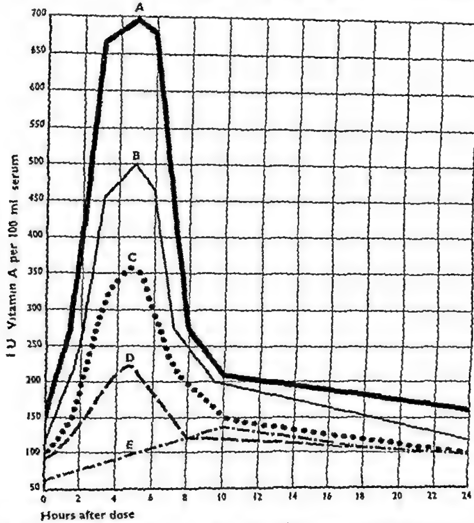


FIG 6 Vitamin A absorption curves the vitamin being given orally dissolved in oil

A Male adults after 250 000 I U
B Female adults after 250 000 I U
C Children after 50 000 I U
D Children after 25,000 I U
E Cœliac disease after 50 000 I U

the beginning The same type of curve has sometimes been found in ulcerative colitis [244], while the types of curve found in diseases which affect mobilization are discussed later

All these flat curves, with the probable exception of those in intestinal tuberculosis, become virtually normal if the vitamin is given in an aqueous dispersion (p 18) while in cystic fibrosis of the pancreas absorption is normal from oil if vitamin A alcohol and not the natural ester is given (p 17) In nephrosis [226] the curve is at a startlingly high level, thus in one very severe case the fasting level was 1 359 I U, which rose after 6 000 units in oil per pound of body weight to 3,197 units in three hours, to 8,365 units in six hours, to 8 591 units in twenty four hours and was still 6,094 units in forty eight hours It must again be emphasized, as it has been on p 17, that

absorption curves give no information as to the total amount of vitamin A absorbed

Intramuscular injections of vitamin A in oil though physiologically as active as by mouth (p 17) cause no rise in the level in the blood [162 222 230] because absorption from the site of the injection is so slow, thus Masi [222] showed that in guinea pigs such an injection caused a steady increase in hepatic stores up to the sixtieth day, though during sixty days

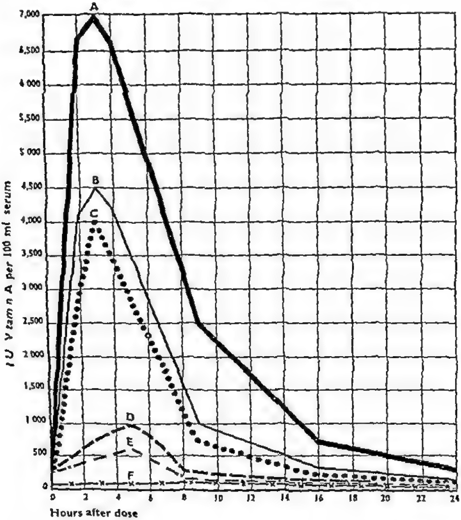


Fig 7 dissolved in

A
B
C
D
E
F

after the same dose had been given by mouth stores steadily fell to a low level He therefore suggests that it is better to give single large doses of vitamin A by injection rather than by mouth since the effect is more prolonged Injections of an aqueous dispersion (p 18) of vitamin A are painful they cause a very slight and delayed rise [162]

The Mobilization of Hepatic Stores The balance between *storage* and *mobilization* must play an essential part in the maintenance of the level of vitamin A in the blood since the liver is virtually the only storehouse of the body But how such mobilization is controlled is obscure Glover (p 21) has suggested that the true hepatic cells contain an enzyme [610] which

converts the stored inactive esters into the active alcohol, and it is the level of this alcohol in the liver which keeps the alcohol in the blood constant (p 24) Many factors are known which affect this production of the alcohol or, at least, the maintenance of the level of the total vitamin A of the blood

Diet, after a period of normal nutrition, causes mobilization or replenishing of the hepatic stores with no alteration in the blood levels Thus in the rat [151] the level in the blood remains constant until the stores are depleted, and a review of the literature [93] suggests that, when levels in man have fallen very rapidly because of a deficient diet, this has been due to low reserves being rapidly exhausted This is supported by the work of Callison and Orent-Keiles [238], who showed that while depletion on a deficient diet, as judged by dark adaptation, took from two to six months—an unusually short time [93]—yet depletion of the same subjects could be achieved a second time, after only a short period on a normal diet, in thirteen to sixteen days. Conversely a very high consumption of vitamin A does not increase the level in the blood, a group of nurses, for instance, who took 50,000 I U. daily for nearly two years having no higher blood levels than nurses on ordinary diets [239]

The *individual* tends to preserve a particular level which is characteristic of himself , thus after single large doses of the vitamin his blood level reverts to his normal after about twenty-four hours [220], and even after taking 3 000,000 I U over twelve days the level in the blood is back again to the individual's normal in another ten days [220] Also, deprivation of vitamin A may leave the individual's level unaltered ; for instance, the blood levels in the deprived subjects investigated by the Medical Research Council [93] varied very little from month to month over many months Reports that a diet low in the vitamin may within a week almost halve the vitamin A alcohol in the blood [177] while the ester may fluctuate in either direction [237] or fall [177] may possibly be congruous with the monthly investigations of the Medical Research Council, because after "prolonged periods" of deprivation the ester and the alcohol may both rise [237]

Pregnancy, according to Bryn and Eastman [102] and to Aron [220], who in 1949 reviewed his own work and that of several others, causes a slight fall in the level of vitamin A, most marked in the last trimester , after delivery the level rises to normal within forty-eight hours even when no vitamin A is consumed In other words, pregnancy decreases mobilization from the liver, though individual levels, according to Cayer and others [245], may remain above the average and are related to the level of lipoids in the blood After parturition the alcohol but not the ester rises [216]

Drugs may mobilize stores of vitamin A Thus according to Hoch and Hoch [216] a dose of 60 ml of ethyl alcohol leaves the level of the inactive vitamin A ester unchanged, while after two and a half hours the active vitamin A alcohol has risen by ten to fifteen per cent and is still rising The smallest dose which causes a significant increase is 20 to 40 ml Pett [246] states that in dogs ethyl alcohol quadruples the level of vitamin A after forty eight hours, while Clausen [251] states there is a considerable rise in the ester Somewhat similar results have been recorded for man by Clausen [501] and several workers whose papers have been summarized in the Medical Research Council's report on vitamin A [93] though the original work described in this report failed to show that vitamin A has any effect what soever on the level of vitamin A in the blood Even so the balance of evidence is heavily in favour of alcohol mobilizing vitamin A

Adrenaline and stimulation of the greater splanchnic nerve has been claimed to raise the level of vitamin A in the blood of rabbits [247] but Goodwin and Wilson [248] could not confirm that adrenaline had any effect in either rabbits or rats, and work purporting to show that adrenaline mobilizes vitamin A in man is most unconvincing [249] Clausen [251] found that adrenaline, insulin and mecholyl chloride all had no effect in rabbits

Illnesses which interfere with the absorption of vitamin A such as those mentioned above when discussing tolerance curves all tend to cause low fasting levels though these are often within the lower normal limits for instance Kramer and others [162] found the normal for children was 135 I U and the average for five children with cœliac disease was 117 I U In nephrosis the fasting level is high or very high for instance, levels of 1 665 I U have been reported [226] the latter being found in the severer cases with the higher lipæmia though there was no quantitative relationship between the degree of lipæmia and the level of vitamin A It is the ester which rises in nephrosis [177] why it does so is obscure though from the very slow fall in the absorption curve (p 6) it is obvious that hepatic storage is grossly impaired In nephritis on the other hand the alcohol rises [177], this may be due to increased mobilization occurring to counteract the excretion in the urine—which is discussed later

Many other illnesses often because of the associated fever discussed below cause a low level of vitamin A in the blood Spector and his collaborators [167] in 1943 gave an excellent review of their own work and that of others, in acute and chronic infections the serum vitamin A is low and cannot be raised by giving vitamin A by mouth and this was also found in an infant with infected adenoids until the adenoids were removed in children with uncompensated mitral stenosis and also in children with eczema or asthma and hay fever Even feverish colds halve the level of the vitamin [230] Popper [177] in 1948 stated that vitamin A alcohol falls in cardiac diseases malnutrition severe illness phthisis hepatitis of all kinds and especially in cirrhosis and pneumonia though after the latter a rise occurs The ester may rise or fall in cirrhosis [177] The level of vitamin A in skin diseases is discussed on p 65 and in diseases of the thyroid on p 45

Diabetics in the early days of insulin used to be given a very restricted diet the only foods they were allowed to satiety being green leafy vegetables These were eaten in huge amounts in an effort to stay their gnawing hunger The result was that the level of carotene in the blood was often very high [98 240] which led to the idea that diabetics were unable to convert carotene to vitamin A this being supported by the poor dark adaptation said to be found in diabetics [240] But Kimble and her colleagues [241] in 1946 examined the levels of carotene and of vitamin A in the blood of 116 diabetics and while finding that the levels of vitamin A tended to be low they did not find high carotene levels nor did they find that the level of vitamin A was low when that of carotene was high as would have been the case had there been any inability to convert carotene into vitamin A Further the large stores of vitamin A found in English diabetics (p 22) suggest that neither absorption of vitamin A nor its formation from carotene is impaired Probably the correct explanation of the earlier observations on diabetics is that their carotenemia and yellow skin was normal for the large amounts of carotene consumed (p 78) that the low levels of the vitamin and the high levels of carotene in children [98] were due to the normal difficulty all children have in converting carotene to vitamin A combined with a diet probably deficient in fats containing vitamin A that the impaired dark adaptation which was said to respond to vitamin A only as long as it was given and never to

ed mobilization since vitamin A once

a transitory effect were there a genuine

a blood level set too low In support of this is the low levels found in diabetics [241] Of course dark adaptation would be impaired both by hypoglycæmia and hyperglycæmia which means that any studies on dark adaptation in diabetics need the control of many complicated factors which was not done in the cases mentioned so that the importance of the findings must not be over emphasized In other words diabetics like everyone else need a diet containing ample vitamin A but while they may have impaired mobilization they do not have difficulty in

converting carotene to vitamin A nor have they the particular need for it which they have for the water soluble vitamins

Hepatic disease when it is chronic causes low reserves of vitamin A (p ?) and low total levels in the blood [199 201 202] at the expense of the alcohol [177] but when there is acute hepatitis though the level falls [200 201 202 252] again at the expense of the alcohol [177] reserves remain normal [58 201 202 252] After a large oral dose of vitamin A in oil a large amount may be lost in the faeces or all of it may be absorbed [252] There generally is a flat absorption curve [160 166 167 200 220]—though not always [252]—which Aron [220] holds is a very delicate indicator of hepatic function appearing several days before the onset of jaundice and reverting to normal several days before it fades But Harris and Moore [252] found there were too many fluctuating factors—temperature absorption storage mobilization—in infective hepatitis for vitamin A absorption curves to give definite evidence about hepatic function though they did find that the levels of vitamin A tended to improve with improvement in the hippuric acid tolerance test and the prothrombin value Aqueous dispersions of vitamin A are absorbed normally in obstructive jaundice [160]

Fever depresses the level of vitamin A in the blood whether it is caused by drugs [98 231] or by infections This may partly be due to its affect on absorption (p 17) but it must in essence be due to impaired mobilization since after the fever abates the level in the blood returns again to normal or above without any vitamin A having been given this has been confirmed for fever from drugs [231] from typhus [233] from rheumatic fever [234] and from pneumonia [235] Since fever has this effect on vitamin A levels it may well be that the prevalence of night blindness in consumptives [232 236] is not due to an inadequate intake of the vitamin but to impaired mobilization

Levels in the cerebrospinal fluid and in exudates and transudates of the body are of no practical importance Abderhalden and Elsaesser [242] could find no vitamin A in the cerebrospinal fluid even after dosing by mouth while Tomaszewski and Dzialoszynski [243] could only find traces when using larger amounts of the fluid than those normally obtained by lumbar puncture The latter workers also report that they found up to 30 I U per 100 ml in 21 of 25 specimens of pleuritic fluid in 12 of 13 specimens of ascitic fluid in 2 specimens of pericardial fluid and in 2 of 6 specimens of amniotic fluid The presence of vitamin A in these various fluids was not related to their protein content nor to the presence of vitamin A in the urine

Excretion and Destruction Lawrie Moore and Rajagopal [183] in 1941 reviewed the work on excretion of vitamin A by the kidney and also gave the results of their own research The following is chiefly based on their paper Vitamin A is *never excreted by man during good health except possibly* as a breakdown product [212] but it may appear in the urine during illness being found most frequently and in the highest concentrations in pneumonia A daily output of 3 200 I U has been recorded which is said to cease abruptly with the crisis In chronic nephritis vitamin A is common in the urine though in smaller amounts than in pneumonia Still smaller amounts have occasionally been reported in chronic infections rheumatic fever skin diseases diabetes pernicious anaemia asthma cancer normal pregnancy and also

in human urine is ... is always associated ... t all ... ntain the vitamin even though they are capa... taking it up from halibut liver oil Apparently ... the urine is dependent on a functional abnorm... capacity for absorbing or retaining the ... between the liver and ... blood and kidneys wil... to retain vitamin A

marked degree In nephritis the damage to the kidney [illegible] leads to an accumulation in the blood of substances which in [illegible] the solubility of vitamin A and so partially hold it back from excretion

The healthy dog constantly excretes vitamin A [illegible] to note that the level of vitamin A in the dog's blood may be [illegible] without causing any signs of a deficiency [253] and that its [illegible] appears to differ from that in other animals (p 50) Rats never [illegible] vitamin A even when taken in toxic amounts [123, 183] or when their [illegible] damaged by lack of vitamin E [207], and rabbits only when [illegible] The occasional presence of vitamin A in the faeces is presumably [illegible] incomplete intestinal absorption (p 17) Secretion in the milk is [illegible]

The large stores which accumulate during a diet [illegible] are depleted with great rapidity if the diet becomes deficient [illegible] 212 [251] or in vitamin E [206], the depletion being greater [illegible] suggests that [illegible] on the [illegible] of the [illegible] depending on some mechanism for destroying vitamin A [illegible]

[illegible] Vitamin A [illegible] that child [illegible] reverse of the [illegible] vitamin A [illegible] the requirements, being proportional [illegible] Thus Irving and Richards [255] note [illegible] than young rats both to prevent deficiency [illegible] at autopsy, and to keep the development of the [illegible] growing incisor teeth normal Guilbert and his co workers [illegible] cattle, sheep and swine [256] and also in rats [257] the minimum [illegible] agreement with [illegible] requirements based on clinical work (p 58) Other workers [266, 267] using rats have shown that three to four times these minimum amounts throughout life increase longevity and health and delay the onset of senility Guilbert and his co workers also state that the requirements are not affected by the amount of energy expended by the animals, their work being confirmed both as regards the effect of weight and increased metabolism by Guerrant and others [258], who found that when rats on a deficient diet were made to exercise they gained less weight but developed less severe deficiency symptoms than control animals These results confirm Wolbach's [259] suggestion that vitamin A is necessary not for the metabolic activities of the cells, but for the maintenance of the structure of the cells, so that the requirements of the body would depend on its weight, that is its number of cells, and not on its activity The changes in metabolism resulting from a low protein diet [207] and a high fat diet [261] do not influence, respectively, storage and consumption while thyrotoxicosis in man (p 45) appears to increase storage Birds [290, 291] appear to need five to ten times as much vitamin A as animals

All these investigations which run counter to the belief that the young need more vitamin A than the old have admittedly been done on animals, but it is generally believed that the effect of vitamin A is broadly the same in all species [260] so it seems most probable that increasing age and so increasing weight raises the requirements of vitamin A, but that youth, exercise and a raised metabolism have no effect

Sex Sex does not greatly influence the requirements of vitamin A since in countries where xerophthalmia is common both sexes are equally affected [262], and in England Harris [263] found poor dark adaptation equally

common in boys and girls between the age of eleven and twelve though between the age of twelve and thirteen girls were slightly less affected than boys Coward [264] after many years work on rats has shown that the male requires more vitamin A than the female and she suggests this may explain the higher mortality among human male than female infants The less uniform requirements of the female rat [68] may possibly be related to the fluctuating vitamin A metabolism which occurs in the ovaries at least of women [182] Depletion of hepatic stores of vitamin A occurs more rapidly in the male rat than the female [265]

Illness If the list of the amounts of vitamin A stored in the livers of patients dying from various diseases is referred to on p 22 it will be seen that in some diseases—notably in chronic genito urinary conditions chronic sepsis infections of the lungs and alimentary tract and hepatitis—there are low reserves of vitamin A which suggests that whatever is the cause (p 23) the requirements of vitamin A are increased Some diseases hinder absorption (p 17) or cause excretion in the urine (p 30) or impair mobilization (p 27) and so in these large amounts of vitamin A possibly in the form of an aqueous suspension (p 18) may be continuously required if it is desired to keep the level in the blood at a normal level

Poor Utilization of Carotene When discussing the absorption of carotene and its conversion to vitamin A (p 13) it was seen that many factors impair the value of carotene to the body

Action of Vitamin A in the Bo

is the fundamental part played by vitamin A in the metabo
the body all that can be said is that a consideration of its chemical structure with five unsaturated bonds (p 3) suggests its probable function as an oxidation reduction catalyst *while the needs of the body depending on weight and not on metabolic activity seem to imply that it is chiefly necessary for cellular structure rather than function French work on*

...tion of vitamin A it corrects

and also causes disturbances

it in the diet [269]—but such effects can have many explanations other than *those concerned with the* fundamental action of vitamin A itself There is some evidence that vitamin A has a specific effect on the growth of animals [69] and quickens *growth and* prolongs the life of tissue cultures [270 271] which is reminiscent of work showing that a very plentiful supply of vitamin A *throughout the life of the* rat increases longevity and delays the onset of senility [266 267]

The Effects of Lack of Vitamin A on the Epithelial Surfaces of the Body

The specific tissue change due to deprivation *of fat soluble* vitamin A is replacement of various epithelia by stratified squamous keratinizing epi
t ... Wolbach and Howe [272]) *If this statement is broadened to*

the secondary result as was suggested by
the nervous system which also occurs the subject is discussed fully on p 42

The change in the epithelium is the same *in all areas where it occurs* the
... becoming undermined by stratified epithelium which
layer of cells in many places at the same time
owever are not changed maintaining their individual properties so that when vitamin A *is given to a deficient animal* the basal cells promptly start to replace the stratified epithelium with epithelium of the correct type

In considering in detail the changes induced in the various surfaces of the

body Wolbach and Howe's [272] description of the rat will be followed, references being given where later work has amplified their observations, or extended it to other species, whilst post-mortem observations in man are described on p 78. The effects of a deficiency in birds will not be given in detail, as these were reviewed in 1950 by Hogan [290]; they include failure to grow, changes in the nervous system (p. 42), changes in the nasal epithelium with a nasal discharge, degeneration of the nictitating membrane, ophthalmia, pustules in the mouth and œsophagus and deposits of urates.

Young animals develop symptoms first, due probably to their naturally low stores of vitamin A (p. 10), the most stri[illegible] of death being [illegible]

Gl[illegible] before that [illegible]unced. The shedding, however, of desquamated cells by the new keratinizing epithelium into the ducts blocks these with the result that retention cysts occur in the glands, and then secretion is impaired even when the secreting cells are not yet affected. This accounts for the early observations on the common occurrence of abscesses or cysts at the base of the tongue, which are really salivary retention cysts that may become infected. As well as all the salivary glands the conjunctival and lachrymal glands are involved soon and constantly, while the glands of the duodenum are only slightly affected and the pancreas escapes apart from some late changes in the duct In Wilson and Du Bois' [273] child, however, the pancreas was more affected, the ducts being blocked and the acini cystic, though the islets of Langerhans appeared to be normal (pp 36, 47) There is late atrophy of the sebaceous glands in the rat [275, 276], but in man they and the sweat glands may become blocked early in the deficiency (p 65).

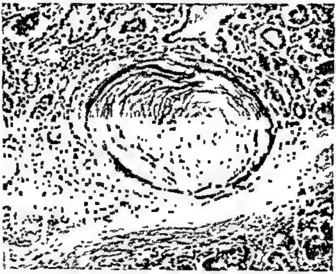


FIG 8 Pancreas of an American infant showing duct occluded by keratinizing epithelium

Air Passages All the respiratory tract, the nasal sinuses, and Eustachian tubes are involved This is of special importance in the lungs, where the smaller bronchi become plugged with desquamated cells, thus paving the way for bronchiectasis and infection, both in animals and man (pp 36, 78). The otitis media, however, which is usual in deficient rats does not occur in children [277]

The Eyes. The conjunctiva undergoes constant and early keratinization followed by œdema and opacity of the cornea and its invasion by blood vessels from the sclera [272, 305] Dryness or xerosis of the eye is caused by the drying up of the lachrymal secretion due to the involvement of the lachrymal glands. The latter was held at one time to be the primary cause of the changes in the conjunctiva and cornea, but Wolbach and Howe [272] showed that xerosis occurred after keratinization, which is supported by clouding of the cornea in the horse [274] and xerosis in man (p 72) occurring at the same time as an increased secretion of tears. Ultimately the whole cornea softens, and the condition known as keratomalacia occurs which, aided by infection, leads

to perforation The changes observed in man which are broadly the sai are described on p 72

The corneal vascularization which follows the keratinization is said Bessey and Wolbach [278] to be indistinguishable in the rat from that wh occurs with a deficiency of riboflavine (p 316) but Bowles and others [3 after very careful work believe that there is a slight difference with lack vitamin A vascularization tends to be more dendritic with a denser col of blood vessels round the cornea But if there is really no difference t raises the very interesting question whether the corneal vascularizati caused by lack of vitamin A is not really directly due to a local deficiency riboflavine caused by the absence of ocular secretions since it appears probal that the cornea is normally supplied with riboflavine not by the limbic blo vessels but by being bathed in the tears and Meibomian secretion both these containing large amounts of riboflavine [281] In any case corn vascularization is a sign of little value since it also occurs in rats [305] a deficiency of tryptophane lysine methionine protein zinc or sodium a from excess of tyrosine from poisoning with thallium and from physical chemical trauma The rather scant descriptions of the vascularization wh occurs in human xerophthalmia appear to have been made when a second infection had blurred the picture [279 280]

Olfactory Epithelium Loss of smell is a late but constant finding in r and other animals [277] though the olfactory epithelium appears to normal [272] The olfactory nerve endings themselves may be damaged osseous thickening of the cribriform plate (p 43) Milas [282] found that t olfactory area in the steer was rich in carotenoids and vitamins A_1 and . which suggests that vitamin A may play a part in smell analogous to that plays in vision It would be interesting to know if a deficiency of vitamin in man causes loss of smell

Digestive Tract The mucous membrane of the digestive tract of the r is not involved apart from slight changes in the œsophagus unless t deficiency of vitamin A is very prolonged when considerable thickening the mucosa of the forestomach occurs which does not persist after t deficiency is removed nor cause the formation of malignant tumours as w once thought [65 283] In dogs and rats a deficiency does not alter t secretion of acid in the stomach nor have a definite effect in dogs on t emptying time [284 285]

In man (pp 73 77) severe deficiencies often cause diarrhœa and the is some evidence that vitamin A is important for the function if not t structure of the gastric glands and pancreas Foldes and Vajda [28 following up unimpressive German work report that in twenty cases wi deficient or absent hydrochloric acid twelve improved both as regards th symptoms and the amount of acid in their test meals after two or thr weeks on 16 000 I U of vitamin A thrice daily The patients who benefit had chronic gastritis neurasthenia gastroptosis diabetes thyrotoxicosis renal lithiasis The eight patients who did not benefit had gastric carcinom pernicious anæmia or gall stones One patient in the last group was giv bile salts as well as vitamin A which caused a return of hydrochloric acid the stomach even though histamine injections had failed Herfort [287 however did not find that vitamin A had any effect in hypochlorhydri though it did increase the amount of lipase and the tryptic activity of pa creatic juice not only in normal subjects but also in those with various vag intestinal symptoms in the latter diarrhœa was decreased and the appeti improved Preliminary work by Seelig [288] suggested that large amoun of vitamin A rapidly removed both the symptoms and radiological gastroscopic evidence of gastric ulcers but work by Douthwaite [289] did n confirm this

Gums and Teeth In man lack of vitamin A causes the gums to becom hyperplastic and keratinized [292] while the developing teeth both

rfants[293] and animals are severely damaged s ... l organ being
of epithelial organ shrinks or is replaced by k ... th lium thus
apparently removing from the odontoblasts s ... influence over
their growth They therefore form poor ... which m
prolonged mild deficiencies leads to the form ... s and tooth
reduplication in the perpetually growing incisor ... such
teeth also lose their colour [296] Mellanby [2 ... m animals
lack of vitamin A in the maternal diet can ... teeth of the
young before their birth It therefore seems pr ... A is the
most important vitamin for the structure of th ... in spite
of the commonly and quite erroneously held b ... in D is
the main cause of dental degeneration (p 570

Urinary System Vitamin A plays an imp ... ction of

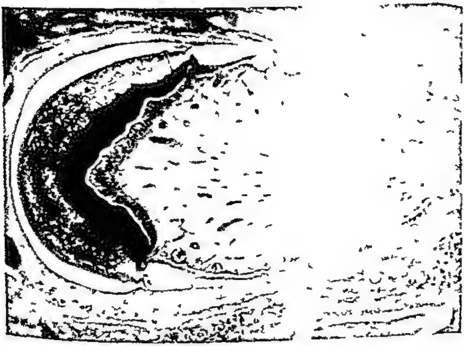


Fig 9 Incisor tooth of rat on a vitamin A deficient diet for 10 days followed by 14 days with addition of butter fat ... inclusions are seen in the dentine Newly formed dentine has filled space between the folds of dentine and has surrounded the inclusions of ameloblasts The restoration of odontoblasts is shown and heavy calcification of the dentine on the labial side of the tooth a usual response to restoration of vitamin A to the diet

the kidney, which is discussed on p 47, but lack of vitamin A does not structurally alter the epithelium of the renal tubules though it causes the usual epithelial changes in the pelvis of the kidney ureters and bladder in animals, in man changes are seldom so severe (p 78) There is however one important result of the change in the mucosa since the new epithelium sheds cells into the urinary tract, so providing a nidus round which salts are deposited to form calculi this occurs frequently in rats and guinea pigs

In man however, it is probable that lack of vitamin A is not a factor in causing renal calculi, though this has frequently been suggested partly from analogy with animals, partly from observations that patients with renal calculi often have symptoms of a deficiency of vitamin A Thus Long and Pyrah [297] and others [298] have reported that renal calculi are frequently though not always associated with poor dark adaptation the most chronic cases having the worst adaptation [297] On the other hand Jewett and his colleagues [299] compared a group of twenty patients suffering from renal calculi with a group of forty normal people, both groups had equally good

c—2

dark adaptation and similar levels of blood vitamin A. Post mortems on seventy eight patients with renal calculi showed no epithelial changes suggestive of a deficiency of vitamin A in either the lungs or urinary tract. It would appear most probable that when urinary calculi and signs of a deficiency of vitamin A occur together it is not the deficiency which has caused the calculi but the calculi which have caused the deficiency through disturbing the metabolism of the vitamin as a secondary result of the damage they have inflicted on renal function (p 47)

Genital Ducts and Epithelium Mason [300] in a very careful study on rats has shown that vitamin A is essential for the germinal epithelium of the testes its deficiency causing changes unlike those produced either by starvation or deprivation of vitamin E. The earliest changes are sloughing of germinal cells into the lumen of the tubules with a gradual reduction in the latter's size. As the degeneration becomes more advanced only three or four layers of cells are left lining the tubules, but these still are capable of forming an occasional sperm and at no time can the testis be so damaged that it cannot return to normal when the vitamin A deficient diet is stopped. These changes are due to a direct effect on the cells themselves and not an indirect one from vitamin A acting on the pituitary since neither pituitary transplants nor injections of pregnancy urine hastened recovery, and also because the degeneration caused by removal of the pituitary is unlike that caused by a lack of vitamin A. A rather puzzling relationship was also noted between vitamins A and E. When both were deficient the testes sooner showed signs of a vitamin E deficiency than when a deficiency of vitamin E was present alone though the decrease in the number of cells due to lack of vitamin A might have been expected to decrease the need for vitamin E. It was further found that a vitamin E deficiency when superimposed on an existing vitamin A deficiency did not cause such serious damage as when the vitamin E deficiency occurred alone. Wolbach and Howe [272] also noted œdema outside the basement membrane of the seminiferous tubules and the usual epithelial changes caused by the lack of vitamin A in the mucosa of the epididymis prostate and seminal vesicles and in the female in the oviducts uterus and vagina. Similar testicular changes have been produced in mice [611] and bulls [301]. The vaginal changes have been used as a guide when doing biological tests for vitamin A being among the earliest signs of a deficiency in animals (p 7) while Hohlweg [302] reports *that in female* infants the appearance of cornified cells is one of the first results of a deficiency. The part played by vitamin A in reproduction is discussed on p 47

Endocrine Glands The structure as apart from function (p 46) of the endocrine glands is said to remain normal no change beyond decrease in size was noted in the rat's anterior pituitary thyroid thymus parathyroids suprarenals islets of Langerhans ovaries Graafian follicles corpora lutea and interstitial tissue of the testes. In a human infant dying from lack of vitamin A Hassall's corpuscles were found to be enlarged [273]

Skin and Hair The rough coat of the rat [275 276] and horse [274] and toad skin and absence of sweat in man (p 67) are due to hyperkeratinization of the epidermis and the atrophy of the hair follicles and the sebaceous and sweat glands and their blocking by desquamated cells. In man the scalp hair and nails are affected little if at all [262 303 304]

The Secondary Result of Changes in the Epithelia due to Lack of Vitamin A *Decreased Local Resistance to Infection*

from the eral ha hat defence mechanisms o
the name anti infective vitamin is too broad in its implications *vitamin*

A is only "anti-infective" to the extent that when it is given to man or animals suffering from its deficiency it increases the power of the epithelial surfaces to resist local infection by bringing them back to their correct and normal condition

The importance of vitamin A for the local epithelial defences of the body has been recognized for many years Among earlier workers Cramer and Kingsbury [316] in 1924 pointed this out very clearly and emphasized that these local defences were not entirely concerned with bacterial infections, since their animals also were heavily infected with intestinal worms, which is supported by vitamin A deficient rats being more susceptible to infection with trichinosis [97] and deficient chicks and turkeys being more susceptible to coccidiosis and trichomoniasis [290] Green and Mellanby [10] found that rats on a vitamin A deficient diet all died with mucosal infections, and that the addition of vitamin A, as carotene, to the diet afforded a degree of protection against these infections which was proportional to the amount of carotene added [317] That the value of vitamin A is purely due to its local effects is shown partly by the infective lesions caused by its deficiency being always epithelial, and partly by observations on the relationship of the humoral defences of the body to vitamin A Thus Gellhorn and Dunn [318] found that the phagocytic index during the early stages of a deficiency might be increased or decreased, but that after a prolonged deficiency it was always low ; they suggest that when the index is raised it is due to the normal reaction to infection, and that when it is low after a prolonged deficiency this is due to exhaustion and is not a direct result of the deprivation of vitamin A, though the index being low sets up a vicious circle which further decreases resistance Torrance [319] observed that vitamin A deficient animals were no more susceptible to bacterial toxins than were normal animals, and it is the antibodies in colostrum and not the vitamin A which protect calves against white scour [336] In ducks [337] lack of vitamin A has no influence on the course of *Plasmodium lophuræ* infections

Changes in the blood picture due to deprivation of vitamin A are not so severe as to suggest they would seriously decrease resistance to infection Wagner [320] studying ten men who took an experimental diet nearly devoid of vitamin A, noted a decrease in the hæmoglobin and erythrocytes, degenerate red cells, a leucopenia with degeneration of the myeloid cells, and a marked fall in the thrombocytes, though none of these changes except the latter were very marked Abbott and others [321] who diagnosed vitamin A deficiency in 84 children 45 women and 28 male students by the condition of their skin and conjunctivæ, and by their diets, found a mild leucopenia with a decrease in polymorphs, a relative increase in large lymphocytes, a decrease in small lymphocytes, with an increase in juvenile and degenerate cells [illegible] previously obtained in vitamin [illegible] were due to lack of vitamin A [illegible] patients returning to normal after they had taken 51,000 I U daily for six weeks Sweet and K'Ang [262], however, in their very extensive study of vitamin A deficiencies among the Chinese found no alteration in either the red or white cells of the blood, and Hennessey [334] in Uganda reported that in prisoners who were deficient in vitamin A the giving of cod liver oil did not alter the leucocytic response to injections of a bacterial antigen The bone marrow of vitamin A deficient rats is normal [335]

Clinical work on the whole bears out that vitamin A is only of value for increasing resistance to infection when the patients are on a deficient diet and the infection is chiefly concerned with epithelial surfaces , but observations on man are difficult to interpret since there are few reports which accurately mention whether the patients treated with vitamin A were previously on a good or deficient diet In the first important clinical trial of vitamin A Green and others [322], having observed that local infections of

the uterus and Fallopian tubes developed in vitamin A deficient rats after parturition gave 275 pregnant women in the last month of pregnancy—when they were presumably deficient in vitamin A (p 65)—large supplements of

Fig 10 Uterus of an American infant with epithelium in part replaced by keratinizing epithelium

vitamin A as "radiostoleum" Only 1 1 per cent developed puerperal sepsis, as against 4 7 per cent of 275 women who had had no extra vitamin A

Fig 11 Lung of a n h ectatic cavity due to keratin The cavity is partially lined bronchi keratinizing epithelium

Donaldson and Tasker [323] in Johannesburg reduced the mortality from pneumonia among native workers from thirteen per cent in 100 untreated cases to eight per cent in 200 cases who were treated with extra vitamin A in the form of "radiostoleum" or liver Orenstein [324] failed to confirm these results

Ellison [325] found vitamin A of some slight value in measles though Mackay and others [326] could not confirm this Sutliff [329] failed to protect children with scarlet fever from developing otitis media by giving them vitamin A In typhoid fever in children Giraud and Valette [328] state that vitamin A is of great value for preventing hæmorrhage or perforation of the bowel and skin lesions but it has no effect on the cardiac and pulmonary complications or the duration of the disease Other forms of enteritis might be benefited by vitamin A since its deficiency in animals increases not only the number but also the variety of intestinal bacteria [338]

The "common cold" in its relation to vitamin A has been investigated

by many workers. Again the rule appears to hold good that vitamin A is only of value when it corrects its own deficiency. Thus Wright and others [330] in Canada found no effect at all from giving large extra amounts of vitamin A to twenty of sixty infants all of whom already received a dessert-spoonful of cod liver oil daily, and Uddstromer [331] likewise observed no [illegible]

vitamin A [332] and some workers have reported that in poorly nourished people the number of colds is also less [333] Vitamins A and D given together are stated to have a greater prophylactic value than either given alone [341]

In infections of the skin vitamin A may be of great value, Ryrie [342] reporting that vitamin A or carotene is almost a specific cure for leprous ulcers when applied locally, and Binyai [343] obtained good results with cod

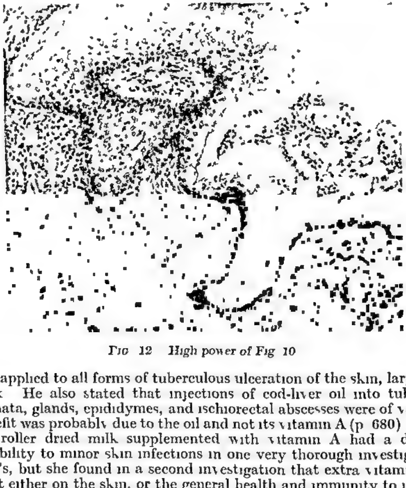


Fig 12 High power of Fig 10

liver oil applied to all forms of tuberculous ulceration of the skin, larynx, and pharynx He also stated that injections of cod-liver oil into tuberculous empyemata, glands, epididymes, and ischiorectal abscesses were of value, but the benefit was probably due to the oil and not its vitamin A (p 680) Infants fed on roller dried milk supplemented with vitamin A had a decreased susceptibility to minor skin infections in one very thorough investigation of Mackay's, but she found in a second investigation that extra vitamin A had no effect either on the skin, or the general health and immunity to infection, from which she infers that some dried milk may be adequate in vitamin A, but that supplementing it is wise [327] Thirty cases of senile vaginitis had their symptoms and the changes in the mucosa improved by Simpson and Mason [345] who gave cod-liver oil by mouth Skin diseases and vitamin A are discussed on p 65

Fœtal Development and Rubella This subject may be of great importance because of the possibility that human congenital abnormalities are due in some cases to the fœtus receiving insufficient vitamins at critical periods of its development If this is so it must be remembered that different abnormalities will arise according to the stage of development reached when the

deficiency occurs Further, such deficiencies must probably be severe to have an effect and also be of short duration if the fœtus is not to be *altogether* destroyed Such a profound but very brief deficiency could hardly ever be caused by simple deficiencies in the maternal diet, but it could be caused by illness suddenly but briefly altering the vitamin content of the maternal blood. For instance, even slight fever greatly reduces the level of vitamin A in the blood (p 30), and so it is reasonable to assume that when mothers contract rubella in the first trimester of pregnancy the fœtus is exposed during the short period the fever lasts to an acute *lack of vitamin A* *It is* known that rubella during the first trimester may cause congenital cataract and congenital heart disease [306] and congenital deafness [307], it is also known—and discussed later—that in sows and in rats a deficiency of vitamin A causes farrows and rats to be born with abnormalities of the eyes and also, at least in rats, with cardiac abnormalities Therefore it is tempting to suggest that it is not the toxin of rubella but its indirect effect on the level of vitamin A in the blood which destroys the nice organization of fœtal development If this be true, then it is valueless to give vitamin A by mouth during rubella, since this will *not raise the vitamin A in the blood (p 30), but large* doses of vitamin A *alcohol* (p 24) should be injected daily in an aqueous *suspension* (p 18)

Hale [308] found that farrows born of vitamin A deficient sows were blind, often with *no* eyeballs, *cleft* palates, hare lips, extra ear like growths and misplaced kidneys *Lack* of vitamin A also causes *congenital blindness* in calves [309], while in rats [310] the commonest defect is replacement of the *vitreous humour by a fibrous retrolenticular membrane, other frequent* abnormalities being colobomas, abnormal structure of the retina, defects of the cornea and other failures of normal development In rats there is also a high incidence of diaphragmatic hernia, especially in breeds where this is common [311], but cystic fibrosis of the pancreas does not occur [311] Misplaced kidneys have been reported in farrows [308] while in rats [312] there may be hypoplasia of the renal parenchyma, ectopic kidneys ectopic ureteric openings, abnormal development of the genital ducts and less commonly etc There is also keratinizing metaplasia iro genital tract The cardiovascular d ɔed and illustrated by Wilson and Warkany [313] who state that "*they are of particular interest because they show remarkable similarity to many congenital cardiovascular conditions that occur in man*" "It is emphasized that present clinical and experimental observations indicate that environmental as well as genetic factors may alter the development of the cardiovascular system" The chief findings in rats were defects in the interventricular and aortico pulmonary septa, abnormal development of the arch of the aorta and of the arteries arising from it, and abnormalities of the *ductus arteriosus* Infants have been born with keratomalacia [314] and it has been suggested [315] that *vernix caseosa* is caused by the hyperkeratosis of a deficiency of vitamin A, but this is probably incorrect as there is no relationship between the amount of *vernix caseosa* on newly born infants and the level of vitamin A in their blood [103]

Vision Vitamin A is essential for scotopic vision, that is, for vision in dim light, it is also probably essential for photopic vision, that is, for vision in daylight, and for the appreciation of colour

Scotopic Vision or Vision in Dim Light Even with a mild deficiency of vitamin A the rapidity of dark adaptation in most people and its extent in all people is impaired [346], while a severe deficiency leads to complete night blindness or hemeralopia The subject is important partly because night blindness is a grave drawback to countrymen or fishermen working in the dusk or by moonlight and to town dwellers during "black outs" partly because poor dark adaptation is a very early symptom of a deficiency which is widely used in nutritional research on vitamin A For a *full* discussion on

the physiology of scotopic vision the excellent review by Lythgoe [347] should be consulted here only enough can be said to explain the commonly accepted role of vitamin A (Clinical applications are discussed on pp 60 and 72)

In bright light vision is carried out by the cones of the retina while in dim light the rods are used When illumination is suddenly decreased so that there is only about as much light as is given by a three quarters full moon vision is impossible for a moment and then dark adaptation occurs the eyes growing accustomed to the dark For about the first six minutes of this adaptation the increasing power of vision is due to an increasing sensitivity of the cones to a poor light but after this further adaptation which may not be complete f
an increase in sensitivity of
the rods of the retina these
can stimulate the cones

The rods however are not directly stimulated by light but only indirectly through the chemical changes light causes in visual purple or rhodopsin which is a conjugated protein found in the dark adapted retina the prosthetic group being derived from vitamin A [348] it is contained in the outer segment of the rod [349] When light falls upon it it is converted to vitamin A through retinene or vitamin A aldehyde by a complicated series of reactions which have been partially unravelled by Wald and Hubbard [349] these are of particular interest because they may involve codehydrogenase I and therefore nicotinic acid (p 339) The improbable possibility that riboflavine is also concerned in these reactions is discussed on p 64 In the intact eye in the dark the vitamin A which has been formed through exposure to light is again conjugated with protein to form visual purple nothing is known about how this occurs except that it probably does not involve the formation of retinene [349]

Since visual purple is thus bleached or destroyed by light the eye contains little after being exposed to bright light and so has to reform it before it can be utilized by the rods for vision in dim light On the rapidity with which the visual purple is reformed must depend the rapidity with which dark adaptation that is full use of the rods can occur Since vitamin A is a necessary part of visual purple any shortage of vitamin A must slow down the formation of visual purple and so slow down dark adaptation Since even the dim light in which rod vision is used destroys some visual purple the amount in the retina will depend on the relative rates of destruction and formation This is the reason for shutting one s eyes for a minute on coming from a brightly lit room to a dark street by shutting out all light the visual purple can accumulate more rapidly In the same way badly fed slaves used to see better in the dawn after a night in the dark than at dusk after a day s work in the light For centuries poorly fed fishermen have known that a day s exposure to glare from the water often causes sudden night blindness—in other words prolonged bleaching destroys so much of the visual purple that the vitamin A deficient eye cannot reform it in sufficient amounts to give even poor night vision On the other hand dark adaptation cannot be improved beyond the normal however much vitamin A is taken [350]

Two other factors besides vitamin A influence the formation of visual purple Firstly there must be an adequate supply of oxygen to the retina and secondly visual purple is regenerated more rapidly—as far as the supply of vitamin A permits—if the retina has been previously exposed for a long period to a bright light The latter fact may be of importance in clinical work on dark adaptation though it appears to be generally ignored

There are many clinical reports that delayed dark adaptation or even severe night blindness can be cured in a few hours with vitamin A while others state it may take weeks or months It is possible that the latter w also having to cure some further defect from the vitamin A deficiency

as nervous degeneration in the retina, or in the rods themselves since visual purple is an integral part of their structure and so, by its absence, might cause structural damage [351]. There is also every reason to believe that dark adaptation is dependent on fine readjustments in the nervous system of the retina once sufficient visual purple has been formed. Thus while the amount of visual purple during adaptation may be only doubled sensitivity may increase 10,000 fold, which appears to be only explicable if a synaptic rearrangement of the nervous elements of [illegible] to become connected to additional nerve [illegible] of subliminal stimuli—a theory which is [illegible] in visual discrimination which occurs during dark adaptation.

Photopic Vision or Vision in Daylight and Appreciation of Colour In sharp contrast to scotopic vision, vitamin A plays no clinically obvious part in photopic vision, since, however great is the deprivation, vision in bright light and the recognition of colours remains unaltered or altered so slightly that it has never been noticed except by Wosika [355], who found some impairment in the recognition of blue On the other hand, there is no substance in the eye apart from vitamin A which could be used for photopic vision, and there is strong evidence that it is so used But if this is correct then the cones must so tenaciously retain their vitamin A and preserve it in so self-contained a photosensitive system that none is ever wasted and no fresh vitamin is ever required In other words, vitamin A must be an [illegible] ucture of the cones, fixed there for ever [illegible] ves that the visual purple of the rods [illegible] of luminosity not only in dim but also in bright light, the cones thus being left with the sole function of mediating colour sensations

Ball and Morton [353] have investigated whether vitamin A or retinene can provide the colour receptor substances postulated by Granit's theory of modulators [354], and they have found that broadly speaking this is so, at least *in vitro*

Vitamin A and the Nervous System. Deprivation of vitamin A causes in animals a primary degeneration of the nervous system and also a secondary degeneration, discussed later, due to pressure from the abnormal or inadequate growth of the skull and spinal column In man the influence of vitamin A on the nervous system is less certain; it is discussed on p 74

Primary degeneration in young deficient rats occurs so constantly that Irving and Richards [72] and Coetzee [73], after very careful work, suggest it should be used as a method of assaying vitamin A, since the difference between the amount o [illegible] system is very small [illegible] present after seven we[illegible] been present for some time before that and since it occurs in rats that show no other sign of deprivation, it must be among the earliest signs of a deficiency. That inanition alone causes no such degeneration has been shown by Aberle [335] and by Wolbach and Bessey [356]

Many other workers, whose investigations were summarized in the second edition of this book, have reported changes in the nervous system of deficient rats and rabbits but since they did not examine the osseous system there is no knowing whether they were observing a primary or a secondary degeneration In young rats [72, 73, 356] both forms of degeneration occur while in dogs, as far as is known, the nervous degeneration is secondary to the osseous changes discussed later In chicks Wolbach [362] reports injury from osseous pressure, but Adamstone [357] f[illegible] evidence of this but [illegible] pinpoint areas of primary degeneration [illegible] stem, base of the [illegible] ebellum optic chiasma and, rarely, in the [illegible] In ducklings [358] the apparent overgrowth [illegible] parts of the spinal cord in the spinal [illegible] of [illegible] Be[illegible]y's rats (Figs. 13, 14) but

the picture is confused by hæmorrhages confined to the upper cervical cord and medulla—in spite of there being least overgrowth and compression here—and also by the formation of true bone in both the white and the grey matter Later work by the same authors [358] suggests nervous degeneration is the essential lesion

Internal hydrocephalus and an increase in the pressure of the cerebro- ... clear Against it being ... observations of Moore

... [366] that in calves papilloedema and the raised pressure decrease rapidly when vitamin A is given This decrease could not in the time have been caused by a return to normal of the cranial bones It seems most probable that the pressure of the cerebrospinal fluid is affected by lack of vitamin A because the ependyma is in origin epithelial and thus dependent, like all other epithelia, on an adequate amount of vitamin A The problem is further complicated by a report [367] that vitamin C also reduces the pressure of the cerebrospinal fluid of vitamin A deficient calves (p 50)

It appears possible that the nervous degeneration is really due to some unidentified factor which is absent in some experimental diets and not others [361], or even more probably, in view of the protective action of vitamin A [72, 73] that the nervous degeneration only occurs when there is a double deficiency of vitamin A and an unknown factor For instance, the work of Wintrobe and others [359] on pigs which in spite of a simplified diet supplemented with nearly every known vitamin still developed a widespread nervous degeneration, shows how complicated and delicate are the dietetic needs of the nervous system even copper is important [360]

FIG 13 Brain of ... compression o... num and nur... sinuses

Vitamin A and Growth of Bone. Our knowledge of ... rol which vitamin A ... lly based on Mel... Bessey's work ... is unfortunate that these investigators do not agree with each other the former, broadly speaking finds that lack of vitamin A alters the pattern of bone growth while the latter

find that growth is largely arrested. *But both agree that the nervous system* is compressed and damaged by the osseous. Mellanby [363, 364, 365] has shown that vitamin A controls the shape of growing bone and especially its fine moulding by influencing the position and the activity of osteoclasts and osteoblasts. A deficiency [illegible] but rather to a slowing or [illegible] or reversal to a lesser or [illegible] especially in the cranial bones which are the most studied by Mellanby, is that there is a general thickening and dysplasia with *loss of the fine* architecture of the bone. This has the disastrous result that the foramina through which pass the *cranial nerves* are narrowed and so the nerves are compressed. Among the cranial nerves those which suffer most are the olfactory, auditory and optic, while in the spine compression is worst in the cervical region; the hind brain also suffers. In deficient adult dogs this bone dysplasia with its subsequent nervous changes may not appear for two years. In rats [365] Mellanby has produced the same changes as in dogs, while in calves [366] lack of vitamin A causes stenosis of the *optic foramina*. When vitamin A is given to deficient puppies there is a return to the normal distribution of osteoclastic and osteoblastic activity which is often intense, apparently being aimed at the restoration of the correct shape of the bones [365]. Irving [638] largely agrees with Mellanby but emphasizes that vitamin A chiefly acts on the osteoblasts which are stimulated by its lack and depressed by its excess.

FIG. 14. Nervous system of young vitamin A deficient rat *showing the disproportionate growth between the nerve roots and spinal canal, leading to herniations* of the former. (The herniations have been dislodged so as to demonstrate them.)

Wolbach [362], on the other hand, believes that vitamin A affects the growth of bone because it is essential for the growth, maturation and degeneration of epiphyseal cartilage cells. *Lack of vitamin A arrests this normal evolution* of cartilage as does inanition from any other cause, be it due to insufficiency of a perfect diet or to insufficiency of only one essential food. But with all forms of inanition except that due to vitamin A the arrest of endochondral bone formation takes place only as part of the uniform arrest of all *bodily growth*. With lack of vitamin A on the other hand growth of bone

ceases *before* that of the rest of the body This leads to compression of the still growing central nervous system, which is convincingly shown in Wolbach and Bessey's most interesting paper [356], from which we have been fortunate enough to be allowed to reproduce two photographs (Figs 13, 14) The brain herniates into the venous sinuses and foramen magnum, the spinal nerves herniate into one or more of the intervertebral foramina which they pass before leaving the spinal canal, the spinal nerves are also kinked into large pits on the dorsal surfaces of the vertebral bodies, these pits being apparently due to the nerves eroding the bone as they are forced into the foramina of emissary veins The spinal nerves which are thus herniated show the classical histological picture of degeneration and regeneration following a crush injury this strongly suggests that this nervous degeneration is not a primary degeneration due to the lack of the vitamin

A further effect of lack of vitamin A according to Wolbach [362] is that remodelling ceases though the deposition of bone by the periosteum and endosteum continues normally It is suggested that remodelling is caused by an agent or "inductor" provided by dying epiphyseal cells acting on "competent" osseous tissue Since lack of vitamin A stops the evolution of cartilage cells, there can be no "inductor" and so no reaction of "competent" osseous and so no remodelling this is supported by the effects of excessive amounts of vitamin A which cause a very rapid evolution of cartilage cells and so, on this theory, an excessive liberation of "inductor" with such great remodelling activity of "competent" tissue that fractures are common owing to the new bone which is laid down not being dense enough to take over the strain imposed by the removal of the old bone The effect of hypervitaminosis A on bone is discussed further on p 85.

Vitamin A and the Endocrine System. *The thyroid* among the endocrine glands has been most fully investigated, though it is still impossible to force all observations into the strait-jacket of one rigid theory Drill [369] has given an excellent review of the literature up to 1943, since when further light has been thrown on the subject, chiefly owing to the use of thiouracil, to the realization that the conversion of carotene occurs in the gut wall and to the use of iodine isotopes It now seems probable that (*a*) the thyroid affects carotene metabolism by increasing its absorption from the gut into the gut wall (*b*) the thyroid plays no part in the conversion of carotene to vitamin A within the gut wall, (*c*) the thyroid does not to any great extent directly increase the body's need for vitamin A and (*d*) vitamin A decreases the effect of thyroxine in stimulating metabolism

Cama and Goodwin [370] in 1949 showed from work on rats and rabbits that hypothyroidism caused by thiouracil increased the fæcal excretion of carotene and did not cause the appearance in the blood of either carotene or vitamin A aldehyde from this it seems clear that hypothyroidism hinders the conversion of carotene to vitamin A by hindering the absorption of carotene into the gut wall and not by preventing its conversion after absorption into either vitamin A or its possible immediate precursor vitamin A aldehyde (p 13) Further, desiccated thyroid decreased the excretion of carotene and when given together with thiouracil nullified the effect of the latter, which shows that the thyroid acts not by stimulating the conversion of carotene to vitamin A but by stimulating the absorption of carotene Cama and Goodwin [370] have explained that as regards rats and rabbits there are no inexplicable discrepancies between their work and that of others which they review, while their own is clearly confirmed by Johnson and Baumann [371] who investigated the effect of thyroxine and thiouracil on carotene metabolism as judged by liver storage of vitamin A The latter authors further report that the effect of thyroxine could not be duplicated by dinitrophenol, so that it is not simply the raised basal metabolic rate caused by thyroxine which is the reason for the increased absorption

Clinically the above work is supported by night blindness in myxœdema

[372] by the low level of vitamin A (which is only slightly raised by giving carotene) in the blood of cretins [373] and also by their susceptibility to respiratory infections That hyperthyroidism increases the absorption of carotene above the normal appears to be borne out by Moore [184] finding in the livers of patients dying from thyrotoxicosis larger stores of vitamin A than were present in any other human livers

Some experimental work however is not congruous with any of the above clinical and laboratory work Thus Pasold and Heidemann [375] confirming earlier work found that the milk of thyroidectomized goats was yellow with carotene but contained no vitamin A in contrast to that of normal goats which contains no carotene but is rich in vitamin A Since normal goats do not absorb any carotene into the blood the effect of lack of thyroxine in these animals must be either to prevent the conversion of carotene to vitamin A or to so damage the lower bowel wall that carotene seeps through it in an area where no conversion of carotene takes place It has also been stated that thyroidectomized guinea pigs [376] store carotene but not vitamin A in their livers

Destruction of vitamin A in the body is not increased by thyroxine to any great extent since besides Moore's findings in man mentioned above he has shown that in rats given lethal amounts of vitamin A the addition of thyroxine did not have a protective effect by destroying the vitamin which some workers have reported but instead hastened the animals death [377] while Logaras and Drummond [378] found that the increased metabolism caused by thyroxine and dinitrophenol increased the storage of vitamin A in the liver However Hemner and others [386] judging by the rate of depletion of hepatic stores believe that thyroxine predominantly affects vitamin A indirectly through growth if either hypo or hyperthyroidism checks growth then stores of the vitamin are spared They also have reported that to a very slight extent—masked by the indirect affect on growth—thyroxine itself hastens depletion This latter action is better demonstrated in chicks [382] where thyroxine and thiouracil respectively increase and decrease the amount of vitamin A necessary for growth

The damping effect which vitamin A has on the activity of the thyroid was first suggested by McCarrison [379] who found that cod liver oil delayed the metamorphosis of tadpoles this has since been confirmed with purer vitamin A preparations Experiments on animals by Logaras and Drummond [378] and many others [374 383] have conclusively shown that vitamin A reduces the increased metabolism caused by thyroxine while Belasco and Murlin [383] have reported that the metabolism of thyroid tissue from animals taking large amounts of vitamin A is decreased compared to that of controls this decrease being even greater if the animals have been given thyroxine as well In fact vitamin A and thyroxine far from being antagonistic actually reinforce each other in their action on the thyroid In severely deficient animals [384] the thyroid is said to be relatively heavier than in normal animals but it takes up the same amount of radioactive iodine though the rate of thyroxine synthesis is decreased

Clinical work on the use of vitamin A in the treatment of goitre is not satisfactory The Mellanbys nearly thirty years ago noted a clinical improvement in patients with exophthalmic goitre treated with cod liver oil which they believed to be due to the iodine in the oil some fifteen years later German workers claimed that vitamin A itself was of value in simple adolescent goitre [380] and in toxic goitres [381] but the vitamin A preparation Vogan which they used contained enough iodine to explain their results However the erroneous claim that vitamin A is of value in the treatment of hyperthyroidism still crops up with depressing regularity For instance Simkins [385] in 1947 published an account of two cases who when treated with 200 000 to 400 000 I U daily apparently responded in a dramatic manner but both these cases were the type which often recovers spontan

cously, while Simkins' very extensive review of the literature can but convince any critical reader that vitamin A is valueless in the treatment of thyrotoxicosis

The level of vitamin A in the blood is no guide to the level of thyroid activity [370]

Fertility is not very dependent on vitamin A The structural changes which occur in the testis of the rat and bull as a result of a severe deficiency have been described on p 36, so that here it is only necessary to emphasize that fertile sperm are st[illegible]ced in small numbers amidst the ruins of the germinal epithelium [illegible] bulls, for instance, are too weak to stand [301] [illegible] A, [illegible] but becomes delayed and [illegible] in severe deficiencies rats refuse to mate and [illegible] of deficiency there is mating but no conception, [illegible] conception, but [illegible]ion of the fœtuses, death of the fœtuses, prolonged gestation The death and resorption of the fœtuses is probably due to the fœtuses themselves being so damaged by lack of the vitamin that they could not continue to develop (p 39) In cows [388] œstrus remains normal and conception occurs even when deficiency symptoms have developed though calves are often born dead The report that excessive amounts of carotene by mouth [389] stop œstrus and a desire to mate in rats appears to receive support from the observation that vitamin A applied directly to the vaginal mucosa prevents œstrogenic cornification [390]

The pituitary has inevitably been held to be the primary gland affected by vitamin A, all other endocrine changes being secondary to this For instance, it is stated that the amount of thyrotropic hormone in the anterior pituitary is low in rats on a high vitamin A diet, and high in rats on a deficient diet [391] It has also been found that the factor in the anterior pituitary which stimulates the growth of the female genital system is increased in vitamin A deficient male rats, but Mason [300] has pointed out that this is a purely secondary effect due to the virtual castration of the male rats by the degeneration of the vitamin A deficient testes (p 36), since deficient female rats showed no such changes in the pituitary's secretion

The principle of the pituitary which stimulates lactation does not appear to be affected by vitamin A, since Williams and others [392] found that the amount of milk secreted by nursing mothers was not altered by varying their intake of vitamin A Kepinov [393] in some interesting experiments on starved frogs found that adrenaline did not accelerate the hydrolysis of liver glycogen to glucose unless vitamin A was previously given Vitamin A apparently stimulates the glycogenic hormone of the pituitary, since it has no effect on frogs after the removal of the latter That the function of the adrenals themselves may possibly be directly affected is suggested by Moore [123] and Popper [58] observing that they sometimes store large amounts of vitamin A Wegelin [394] found that vitamin A checked the loss of glycogen from the liver which is caused by thyroxine, but here it seems most probable that the vitamin was directly decreasing the action of the thyroxine (p 46) and not acting indirectly through the pituitary The changes brought about [illegible] thymus of children by a deficiency of vitamin A appear [illegible]es (p 78), and in rats thymectomy [illegible] does not [illegible] cystic degeneration which occurs in the pituitary of [illegible] cattle [396] is probably a secondary result of compression by the osseous hypertrophy which follows lack of vitamin A (p 43)

Vitamin A and Renal Function The relationship of vitamin A to the kidney is not clear, but there appear to be four possibilities which are worth consideration (*a*) The kidney merely acts as a storehouse for vitamin A (*b*) The kidney destroys or excretes excess of vitamin A (*c*) Vitamin A is necessary for the functioning of the kidney and, secondarily, reduces hyper-

tension. (*d*) Impaired renal function allows vitamin A to leak away in the urine and also causes a toxic condition of the body which hinders it in utilizing vitamin A.

(*a*) The kidney is avid for vitamin A, so that at very low levels of intake there is a higher concentration, though not greater total stores, in the kidney of the rat than in the liver [397]. In the male this concentration is considerably higher than in the female, due either to a true sex difference [398] or to the more rapid growth of the male, since growth appears to increase renal stores [399]. At very high levels of intake the renal stores may exceed those found in the liver of animals on normal diets [123].

But the structure of the kidney, the avidity of the kidney for vitamin A, the effect of growth in increasing renal stores and the effect of vitamin A—discussed below—on renal function, all these point to the kidney actively using vitamin A and not merely storing it passively.

FIG. 15 Pelvis of kidney of an American infant filled with keratinized epithelium.

(*b*) However great the excess of vitamin A in the body no vitamin A ever appears in the urine unless the kidney is diseased (p. 30). Of course it is possible that the kidneys excrete the breakdown products of vitamin A (p. 30), but there is little proof that the kidneys destroy vitamin A themselves, as a first step in its elimination, except the observations of Belasco and Murlin [383], who found that renal tissue from rats on a high vitamin A intake had a slightly raised metabolism compared to that from control animals, from which they suggest an increased effort to destroy the surplus vitamin A. The high blood level of vitamin A often found in nephritis [98] and nephrosis [226] does not appear to be due directly to the lesion in the kidney but to abnormal hepatic storage (p. 27)

(*c*) Our knowledge of the rather surprising effect vitamin A has on the excretory power of the kidney is chiefly due to Herrin [400], who found that in rats on a vitamin A deficient diet the urea clearance fell by twenty-three to twenty-seven per cent., this being a purely functional effect, since not only was the urine normal, but histologically no structural changes were found in the kidneys. Further work on dogs, extended to include inulin clearance, confirmed the work on rats and also showed that excess of vitamin A raised the urea clearance above normal, though this could not be maintained indefinitely [401]. It was thought the effect was due to increased glomerular

filtration, which has been confirmed by Bing [101]. Experiments on man were even more interesting [102]. When thirteen subjects were given 50,000 to 75,000 I.U. of vitamin A daily two showed no response, four had an increase in their urea clearance of from eleven to fifteen per cent., and seven had an increase of twenty-four to ninety-one per cent. The last group belonged to the type whose body weight fluctuates widely and rapidly. No subject showed any significant change in blood pressure or oxygen consumption. ... not be maintained, though in some ... ne hundred and twenty-eight days. ... powerful though transitory diuretic in daily doses of 72,000 I.U. by mouth in patients whose weight fluctuates rapidly.

Hypertension is not benefited by vitamin A, though fish oils may have a favourable effect irrespective of their content of the vitamin. Early ... vitamin A in fish oils which sometimes ... [104] and experimental hypertension ... rats [106] has shown that the hypo... oxidation though this destroys their ... vitamin A concentrates is not related ... le and Bouhls [108]. ... hypertension could ... t respond after two ... 000 I.U. of crystalline vitamin A acetate ...

... of observations on man which show that ... with low stores of vitamin A in the liver ... Long and Pyrah [297] and others [298] that ... calculi are frequently though not always [299] associated with poor dark adaptation, the most chronic cases tending to have the worst adaptation [297]. It is not clear from these ... renal function was ... servations appears ... (p. 30), or that the ... g early "uraemia" ... ene (p. 15), or may ... may cause a toxic ... re the night blind- ... vel of the vitamin ... [226].

... A to Other Vitamins. The balance of evidence, ... often scanty, is against vitamin A being directly concerned with the metabolism of any of the other vitamins; some indirect associations, however, have been proved, such as vitamin E protecting vitamin A against oxidation by vitamin F or the essential unsaturated fatty acids (pp. 15 and 23) and vitamin K [415] and possibly vitamin D [416] ... against the hypoprothrombinaemia of ... hand vitamin A in large doses ... ing. Less certain is the calcif... rachitic osteoid tissues caused ... tion of hypervitaminosis D by vitamin A (p. 534) ... and the anchoring ... Even more uncertain is the relation of vitamin A ... complex; for instance it is reported that ... in pigs [418] and that in ... vitamin A ... vitamin A are increased ... deficiency of aneurine, ... by lack of ... of weight caused by lack of aneurine is increased if small amounts of vitamin A are given ...

Ascorbic acid or vitamin C, however, is the vitamin whose relationship to vitamin A has caused most research and most uncertainty. Thus it has been claimed that vitamin A deficient rats live longer and gain more weight

if given ascorbic acid [414] but this could not be confirmed by Sharman [420], while Mapson and Walker [421] have proved false claims [367, 422] that the rat needs vitamin A for the synthesis of ascorbic acid The same appears to be true for the chick [423] Reports that lack of vitamin A causes low levels of ascorbic acid in the aqueous humour of rabbits [424], and in the blood and cerebrospinal fluid of calves [367] could be better explained by the general impairment of metabolism in sick animals than by postulating a direct relationship between the two vitamins, and the same can be said about the reputed effect of scurvy in reducing hepatic stores of vitamin A in the guinea pig [425]

Vitamin A, Fat and Protein Metabolism Josephs [151] considers that vitamin A has an important and specific effect on the metabolism of lipids, basing this opinion not only on his own very careful work with rats but also on the investigations of others, which he reviews Both in man and animals giving large or toxic (p 81) doses of vitamin A causes a transitory rise in the level of serum lipids, including cholesterol, this rise being greater and persisting longer in animals previously depleted of vitamin A Conversely, lack of vitamin A has a specific effect in reducing lipid levels An interesting exception to this effect of vitamin A is found in the dog whose serum lipids remain normal even after poisoning with enormous doses of the vitamin (p 81) In rats it has been shown that vitamin A has a specific effect in increasing the amount of fat in the body [410, 411]

In illness, as Stannus [409] has pointed out, there is often an increase in the levels of carotene in the blood when there is a lipæmia such as occurs in myxœdema, diabetes or lipid nephrosis and in the latter condition vitamin A also tends to be very high (p 29)

Protein metabolism is also intimately connected with vitamin A, since paired feeding experiments with young rats [410, 411] show that the vitamin has a specific effect in causing protein retention and appears to be used up during protein metabolism, since replacing most of the fat or carbohydrate by protein in the diets of rats deficient in vitamin A retards growth and hastens death [414] Meunier and his colleagues [412], after careful work, state that 20 micrograms of vitamin A are as effective as 150 mg of glycine in protecting rats on a low protein diet against the injurious effects on growth of sodium benzoate Sinc
the body owing
that Meunier's c
This is confirmed by similar work carried out with bromobenzene [413]

THE PROVISION OF VITAMIN A IN HUMAN DIETS

Vitamin A and carotene are very far from being one and the same, but propaganda over food during war and the use in advertisements of such phrases as "provitamin-A" and "the amount of vitamin A (as carotene) in this preparation," have spread the erroneous belief that vitamin A and carotene are of equal value in the diet Actually, unit for unit, more carotene that vitamin A is necessary if all requirements are to be satisfied, and also some stores of the vitamin are to be built up, since the body only utilizes carotene as efficiently as it does vitamin A when there is already a severe deficiency of both (pp 12, 16) For banking vitamin A against any future shortage carotene is wastefully used compared to vitamin A Also if pp 11 and 16 are read, it will be seen that while vitamin A is nearly always well absorbed, only five per cent of the carotene in some vegetables may be absorbed by healthy men and, further, other factors, which do not greatly influence the absorption of vitamin A, may adversely influence that of carotene, such as poor general health of the body and of the bowel, the absence of fat and bile, and the taking of liquid paraffin either alone or in an emulsion So it must be emphasized that if the tables on p 54 are consulted about

the carotene content of a food the values given bear little relation to the amount of carotene which will be absorbed All this means that where possible in health, and certainly in disease, some at least of the vitamin A requirements of the body should be supplied by vitamin A rather than carotene (p 58) This is especially important for children (p 12)

Vitamin A itself is found only in a very few foods, of which the commonest a ... ome fish and fish liver oils
Of these ... widely eaten, and for that
reason it ... in content is not constant
but depends entirely on the diet of the hens and cows Broadly speaking, the more that eggs and milk are produced by "commercial methods" the less their value This especially applies to eggs, which when produced on egg farms have only to conform to the naked eye appearance of eggs, since the fact that they are often too deficient in vitamins for any chick to hatch out of them does not here make the farmer feed the hens properly The old belief that a dark yolk meant a good egg is undoubtedly correct, and explains why people still prefer the "farmyard" egg of their holidays to the pallid yolked egg of their town grocer Sjollema and Douarth [426] have summed the matter up by saying "where poultry have access to pasture they eat enough grass to bring up the vitamin A content of the yolks to a maximum This undoubtedly is often not the case with poultry kept to produce eggs for consumption" Of course the colour of the yolk is due to valueless carotenoids (p 11) but these are a good indication of the content of vitamin A itself, except in the improbable event of the fowls having had very large amounts of cod liver oil [427, 428], when pallid yolks may be rich in vitamin A

Cow's milk and butter are excellent sources of vitamin A, their content again being dependent on the diet of the cows Watson and others [436] found that the vitamin A in milk could be doubled and the carotene trebled by altering the diet, though neither could be increased beyond a certain level which varied with the breed of cow Oldfield [437] states that "one pint of milk from a pasture fed cow may be equal in protective value to two pints from a stall fed cow," and it has been suggested that in England, as in Finland, legislation should be passed to ensure that the diet of cows contains enough carotene to maintain the vitamin A content of their milk at a reasonable level [431] The colour of milk and butter is not a good guide to the amount of vitamin A present, since vitamin A and carotene do not run parallel to each other in milk, but vary in their proportions with the breed of cow, Jerseys, for instance, have a yellow milk which contains nearly twice the carotene of that from Shorthorns, but only half the vitamin A [433] Pasteurized milk, dried milk, etc [432] have the same vitamin A and carotene content as fresh milk even after storage for several months, but sweetened condensed skimmed milk has none Goat's milk contains only vitamin A (p 46) and so is quite colourless, which may in part explain the unreasonable bias against a valuable and sweet smelling food Vitamin A in milk and in colostrum is in the form of the ester [451]

Human milk has never been investigated with the care which has been lavished on many less important subjects, probably because of the difficulties [452] implicit in its investigation Kon and Mawson [452] in 1950 reviewed most of the sparse literature while reporting their own studies based on 2,284 samples of milk They found that the mean vitamin A content of milk in England is 153 I U per 100 ml the small amount of beta carotene in the milk carotenoids [452] only contributing the equivalent of about another 3 I U In America values of about 180 I U seem more common [453 454] The season of the year has no definite effect, but the stage of lactation is extremely important, there being a rapid rise [454] during the first three days from an average of 426 to 594 I U per 100 ml, figures of over 1 000 I U being recorded in America After this there is a rapid fall during the next six to ten days and then a more gradual decline until the eighteenth week,

when values again rise [452] Mean English values [452] at three weeks are 230 I U per 100 ml and at eighteen weeks 143 I U Milk rich in fat has a high content of vitamin A and *vice versa*, while the level of vitamin A tends to rise with the age of the mother, but is not affected by the number of her previous children There is considerable variation in the milk of different women, some secreting three to four times more than others [452, 453]

The factors which govern the level of vitamin A have not been fully investigated it seems probable that a high fat diet causes an increase due to the increased fat of the milk [455], and vitamin A given during lactation causes an increase, but daily doses of 4,000 I U during the latter part of pregnancy have no effect [452], due presumably to the liver storing the vitamin, so that the blood level remains constant (p 27) Given immediately before parturition 240,000 I U in oil cause an appreciable increase in the milk content which is still evident after nine days, while 24,000 I U daily for the first nine days of lactation causes an increase of about seventy per cent Lesher and her co workers [453] report doubling or even quadrupling the vitamin A content with 50,000 I U in oil daily, an effect also achieved by a good helping of liver [452] Other American workers giving a single dose of roughly 140,000 I U in an "aqueous dispersion" (p 18) report rises in the milk of about 855 I U after twelve hours, normal levels not being regained for twenty-four hours or more The latter workers also found that the rise in the milk was almost wholly dependent on the level of the vitamin in the blood vitamin A given in oil having the same effect as in an "aqueous dispersion" if given in large enough doses to obtain the same blood level Kon and Mawson [452] on the other hand found from their studies on women not taking extra vitamin A, that the breast actively secretes vitamin A so that the levels in the blood and milk are not related to each other, the greatest difference occurring just after delivery when blood levels are low (p 28) and milk levels very high Probably the only way of enhancing the value of vitamin A in milk throughout lactation—apart from keeping the blood flooded with large quantities of the vitamin—is to increase the consumption of fat since Salmi [457] in Helsingfors found the impoverished war diet caused a fat impoverished milk, while Deem [455] found that extra fat in the diet caused extra fat in the milk which then increases the level of vitamin A [452] Other factors affecting milk have not been investigated except cursorily [452, 455], though the effect of maternal disquietude on milk yields has been long recognized [458] Consuming carotene does not enhance the vitamin A value of the milk [452, 459]

Margarine in England is under Government control and consists of four main varieties [460] "Special" margarine, "Standard" margarine and Kosher margarine are the only kinds which are allowed to be sold to the public by shops and are provided in restaurants the first two of these by law must contain 450 to 550 I U of vitamin A per ounce The vitamin may all be added as fish liver oil or as fish liver oil and carotene Kosher margarine —suitable for Jews and vegetarians who refuse to eat fish oils—contains 200 to 300 I U per ounce in the form of carotene This almost negligible amount is due to the impossibility of adding more without making the margarine too red to mimic butter The margarine permitted to bakeries, cake shops, etc , contains no vitamins Good butter may, of course, contain about twice the vitamin A of margarine [461]

Butcher's dripping bought in the poorer parts of English towns in July, 1940 when dripping was still both cheap and plentiful, had no vitamin A [440] though it did contain as much vitamin D as summer butter and, of course, had a higher energy value than margarine

Lard although containing no vitamin A, contains some substance which has the same biological action as vitamin A This, however startling, appears certain from the very careful work of Kaunitz and Slanetz [462] on rats in America lard was as effective as vitamin A not only for growth but also

for the cure of deficiency symptoms Confirmation comes from France, where Le Gallic [463] has published a series of papers on the results of giving both mice and rats diets devoid of vitamin A but rich in lard

Fish may contain considerable amounts of vitamin A in their body fat, so that fat fish like eels, halibut, herrings, lampreys, salmon and sardines are valuable, but Pyke and Wright [444] found no vitamin A in twelve brands of tinned salmon and in one "chilled" salmon so that salmon as usually eaten in England is worthless from this point of view. In England also, Bacharach and others [429] showed in 1942 that the average individual daily intake of vitamin A from herrings either fresh, tinned, bloatered or kippered, was only 6 I U.—a lamentable fact, as these fish are often cheap and plentiful the herring fleet could provide three herring a fortnight for everyone [430] Two fresh herring in the summer months, when they are rich –1,700 I U of vitamin A as 537) in A, forming the only nat Lovern [430] in 1944, stated that the total amount of vitamin A in fish and fish liver oils which was provided by the English fishing fleet was 1,862 I U daily per person in Britain and this could be greatly increased

Concentrated preparations of vitamin A are available as ordinary cod liver oil, as concentrated fish liver oils, as "aqueous dispersions" (p 18) of fish liver oils and as synthetic vitamin A Of all these, ordinary unconcentrated cod liver oil is by far the best the diets of children and adolescents and even of adults could be greatly improved by their taking daily two teaspoons, which would roughly provide most of the daily requirements of vitamin A as well as most valuable fat (p 675) and vitamin D (p 540) Both the English [430] and South African [435] fishing fleets could bring back enough fish liver oils to make an increased consumption possible The concentrated oils have the great advantage that they can be taken in tasteless capsules but have the two great disadvantages that the amount of their fat is negligible and that they are so concentrated they can easily cause vitamin A poisoning (p 81)

Carotene and the provitamin A carotenoids are very widely distributed throughout vegetables and fruits As a rough rule it may be said that carotene is always present in association with chlorophyll (the green colour of which masks the red of the carotene) and in yellow vegetables and fruits Thin green leaves, like those of cabbage, spinach and lettuce, are especially rich in carotene, while the bleached stalks of celery and the white hearts of cauliflowers contain little or none , white flour and milled rice are again an example of the loss of valuable carotene with the loss of colour The carotene content of tomatoes was not found to be altered when the plants were grown in eighty seven different nutrient solutions, but growing or ripening tomatoes indoors reduces their carotene by over one quarter [446] Factors influencing the carotene content of plants, including the importance of boron, have been reviewed by Maynard [447] The excellent paper by Graves [91] and p 11 should be consulted for a discussion on the different biological values of carotene from different vegetables carotene in red or yellow vegetables like carrots, is very poorly utilized in comparison with that of green leafy vegetables the latter being thrice as valuable—due possibly to their high content of vitamin E (p 15)

In some tropical and sub tropical countries like the Philippines the Dutch East Indies, Ceylon India, China, the West Indies and parts of East Africa the problem of child blindness due to lack of vitamin A is so widespread and so serious that Fitzgerald Moore [441] in a practical discussion of the whole problem concludes that the only hope of a solution is reinforcing with vitamin A concentrates the local vegetable oils and fats which are eaten by the inhabitants. Of these arachis oil is the commonest and forms an excel

lent vehicle for vitamin A It must be stressed that it is no use introducing alien forms of vegetable fats rich in carotene, like red palm oil, to take the place of the local varieties, because both expense and custom will prevent their use

Effects of Cookery, Storage, Canning, Freezing, Drying and Dehydration on Carotene and Vitamin A. Domestic cookery causes no appreciable loss of either vitamin A or carotene, since neither are soluble in water nor easily destroyed by heat The prolonged boiling of milk—though not rapid boiling or pasteurization—and the slow cooking of vegetables in stews is harmful, but there is little loss of vitamin A from butter during cooking and frying [442] Most fats, however, which are used for frying—especially when they are reheated many times as in "deep fat frying"—develop an "anti-vitamin A" factor when heated which destroys part of the vitamin A activity of foods eaten at the same time [65, 66]. Probably such destruction is not of any practical importance except where foods such as commercial fish and chips are eaten in large amounts by the poor, who are always on the edge of a deficiency of vitamin A. The canning of fish probably destroys some vitamin A [429]. The cold storage of vegetables destroys small amounts of carotene [434] but canning, the ordinary methods of storing apples oranges and tomatoes, and the domestic ways of preserving fresh vegetables and fruits, do not affect carotene nor does drying peas and beans though the slow sun drying of fruit may be injurious Dehydration of vegetables followed by reconstitution and cooking may cause a loss of from nil to seventy per cent of carotene [445] ; losses of course will vary greatly with the various processes, such as blanching, to which the vegetables may be submitted [433] The commercial drying and evaporation of milk and its subsequent storage for a year was found not to reduce its vitamin A or carotene [443] The "band drying" of eggs causes considerable loss of vitamins A and D, but "spray drying"—the method usually employed—has no injurious effect [448] Human food is seldom sufficiently rancid to cause any serious loss of vitamin A [64]

AMOUNTS OF VITAMIN A AND CAROTENE IN FOODS

The following figures come from various papers and from the unique tables of Fixsen and Roscoe [449], which should be consulted if fuller figures about fish liver oils and vegetables are required The figures for the vitamin A content of the flesh of fish are only approximate, since research has been chiefly directed to the amount of vitamin A in the body oils of fish The amount of vitamin A in the edible part of fish has been calculated from the latter's fat content [450] on the assumption that this fat is the body oil referred to by research workers

FOOD	International Units of vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams or roughly 3½ ounces		
	VITAMIN A	CAROTENE	TOTAL ACTIVITY
ANIMAL PRODUCTS			
Bacon	0		
Beef			
Kidney	1,100		
Steak	60		
Suet	600		
Bone Marrow			800
Butcher's Dripping	0		0
Chicken	0		
Heart	0 200		
Lamb	0		
Lard	See p 52		

FOOD	VITAMIN A	CAROTENE	TOTAL ACTIVITY
	International Units of Vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams of roughly 3½ ounces		
Liver			
Calf			52 600–159 800
Pig			12 600–30 700
Ox			12 700–41 800
Polar Bear	1 [illegible]00 000–1 800 000		
Seal (Arctic)	60 000–1 300 000		
Seal (Arctic) Oil	3 000 000–60 000 000		
Whale (Antarctic) Oil	500 000–15 000 000		
Pork	0		
DAIRY PRODUCTS			
Butter American	1 203–1 9[illegible]4	164–410	3 500
Danish Nov.–Jan.			3 380–1 2[illegible]0
Feb.–Apr.			1 080–1 600
May–July			1 840–3 610
Sept.–Oct.			4 180–5 6[illegible]0
English			800–2 000
New Zealand			2 400
Scottish Nov.–Jan.			2 410–1 140
Feb.–Apr.			1 [illegible]20–1 530
May–July			2 560–2 9[illegible]0
Aug.–Oct.			2 990–2 200
Stall fed cows			800–2 900
Goat	900		
Cheese Camembert			3 610
English Cheddar			5 500
Cottage			19[illegible]–[illegible]29
Cream			2 110–2 320
Danish			2 400–3 500
Kraft American			2 160
English			3 070
Swiss			1 970
Parmesan			900
Roquefort			2 500–4 000
Eggs Duck Whole	1 800		
Yolk	2 900		
Hen Whole	4[illegible]0–1 100		
White	0		
Yolk	130–8 800		
Spray Dried	As Fresh		
Margarine Vitaminized	192		See p. 52
Milk Holstein Summer	151	5[illegible]	
Winter	101	43	
Guernsey Summer	8[illegible]	1[illegible]0	
Winter	51	78	
Irradiated	Unchanged	Unchanged	
Pasteurized	Unchanged	Unchanged	
Condensed Full cream sweetened			1[illegible]4–442
Unsweetened			128–3[illegible]5
Dried Skimmed			0
Whole			38[illegible]–1 090
Colostrum			900–2 756
Ewe	20		
Goat	133–224	1	
Sow	90		
Colostrum	21[illegible]		
Human	See p.		
FISH			
Bloaters	28		
Carp	1 020		
Cod Flesh	0		
Liver	[illegible]0 000		
Roe	0		

FOOD	International Units of vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams or roughly 3½ ounces		
	VITAMIN A	CAROTENE	TOTAL ACTIVITY
Fish—continued			
Eel	660 18 500		
Hake liver	2 300 92 000		
Herring			
Canned average	28		
	98 210		
with Tomato	53 105		
Fresh English			
Mar	88		
Aug	753		
Sept	193 238		
Dec	35–98		
Kippers	28		
Mullet	130 1 075		
Oysters	140–420		
Salmon Fresh	4–120		
Canned	0		
Chilled	0		
Roe	0–2 000		
Sardines	4 850 54 000		
Fish liver oils			
Cod (B.P.)	60 000		
Cod (Ministry of Food Cod Liver Oil Compound)	100 000		
Cod (retail)	40 000–400 000		
Halibut	2 000 000 36 000 000		
Tunny	512 000–8 000 000		
VEGETABLE PRODUCTS			
Cereals			
Maize		10 900	
Rice Brown		34	
Milled		0	
Wheat whole flour		102–456	
Wheat whole flour bleached		50 76% loss	
Wheat 85% extraction		50–300	
70%		0	
Semolina		291	
Spaghetti		72	
Vermicelli		0	
Fruits			
Apple		50 90	3 000
Apricot Fresh		1 800–2 300	
Sundried		5 100	
Banana		17	300 500
Black currant			100
Blackberry		15 800	
Cherry		600	
Date (preserved)			80
Fig		380	
Gooseberry		15	
Grape			200
Guava		300–400	
Orange juice		0 2–	
flesh		80	
Pear English		3 260	
Palm fruit flesh		100	
Peach White		880 2 000	
Yellow		3 000	
Dried		450	
Tinned			
Peppers (*Capsicum*		110 1 080	
annuum) Green		3 390 37 700	
Red		60 160	
Pineapple Fruit			50
Tinned juice			

FOOD	International Units of vitamin A or microgrammes of carotene or total vitamin A activity in 100 grams or roughly 3½ oz		
	VITAMIN A	CAROTENE	TOTAL ACTIVITY
Fruits—continued			
Plum		0–230	
Prune dried		1 600–2 500	
Raspberry		130	
Red currant		120	
Tangerine		490	
Nuts and Oily Seeds			
Brazil Nuts		10	
Chestnuts		80	
Ground Nuts		[illegible]	
Hazel Nuts		100	
Olive Oil		0	
Red palm oil			
African		110 000–306 000	
Burmese		44 000–56 900	
Malayan Unripe		24 000–60 000	60 000
Ripe		66 000	190 000
Over ripe		62 000	160 000
Walnuts		50	
Vegetables			
Artichokes Globe		200–400	
Beans French		221–400	
Runner			400
Soya			450–970
Soya flour			
Low fat		70	
Full fat		140	
Beetroot		0	
Broccoli leaves			12 000
Brussels sprouts			12 000
Cabbage			900
Carrot		2 000–9 600	
			1 000
Cauliflower heads		38	
Cucumber		0	
Kale		7 500–20 000	
Leeks		1 000	
Lentils		53–450	
Lettuce		1 500–2 400	3 000
Lucerne dehydrated		3 000–6 000	
Marrow		0	
Onion		25	
Parsley		5 000–30 000	
Parsnip		30	
Pea		130–680	
Dried Green		530	
Split		370	
Potato		28–56	
Radish		3	
Rhubarb		100	
Spinach Fresh		2 630–[illegible] 500	
Tinned			1[illegible] 230
Sweet potato Brown		10	
White		0	
Tomato Whole green		170	
ripe		400	
ripened indoors		170–[illegible]70	
Flesh		14 100–[illegible]	
Juice		[illegible]	
Tinned			4 280
Turnip Greens		10 000–[illegible]0 000	
White		0	
Yellow		[illegible]0	
Watercress			

FOOD	International Units of vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams or roughly 3¼ ounces		
	VITAMIN A	CAROTENE	TOTAL ACTIVITY
MISCELLANEOUS			
Chocolate Milk			
Sweetened			480
Unsweetened			36
Cocoa powder			60
Fungi		26	
Honeycomb			0
Marmalade			4 096
Mushrooms		5	
Yeast			0
			0

HUMAN REQUIREMENTS OF VITAMIN A AND CAROTENE

In Health The latest and now widely accepted broad statement on the requirements of vitamin A was made by the U S A Food and Nutrition Board and the National Research Council [464] in 1948 these requirements are "based on the premise that approximately two thirds of the vitamin A value of the average diet is contributed by carotene and that carotene has half or less than half the value of vitamin A" Adults of both sexes, whatever their employment, and children over the age of thirteen are said to require daily 5,000 I U, which should be increased during the latter half of pregnancy to 6,000 I U and during lactation to 8,000 I.U Children below the age of thirteen require 4,500 I U, below the age of ten 3,500 I U, below the age of seven 2,500 I U, below the age of four 2,000 I U, and below the age of one 1,500 I U These figures as will appear from the following discussion, are parsimonious except for children, because carotene is such an unreliable source of vitamin A (p 12)

With a diet containing plenty of the other vitamins (p 49) *and with no* liquid paraffin or paraffin emulsions used as aperients (p 12), the requirements of vitamin A are only dependent on the weight of the body and not on age or physical activity (p 31)

The correct assessment of vitamin A requirements we believe has been given by *Guilbert, Howard and Hart* [147] who in 1940 not only finally reviewed their own work, but also that of others on human requirements

"The minimum (daily requirements of vitamin A or carotene) for significant storage, optimal dark adaptation and reproduction" are for vitamin A itself 60 I U per kilogram of body weight and for carotene (provitamin A) 200 I U, that is for a man of 11 stone, or 70 kg, 4,200 I U and 14 000 I U respectively For "normal growth, freedom from clinical symptoms, and little or no storage," 20 I U of vitamin A and 40 I U of carotene daily per kilogram are necessary, that is for a man of 11 stone 1,400 I U and 2,800 I U respectively Confirmation of these requirements is provided by the Medical Research Council's investigation [93] carried out on human volunteers the conclusion reached was that 1,300 I U of vitamin A itself is the minimum protective dose, which is almost identical with the figure given above Only 2 500 I U are recommended, however, "to cover individual variations and to leave a margin of safety", this is a low figure compared to Guilbert's apparently due to less importance being attached to the building up of reserves The requirements for carotene are so bedevilled by all the factors which influence its absorption (p 15) that it is difficult to compare the Medical Research Council's figures with those already given but broadly speaking they are in agreement Wagner [320] from carefully controlled experiments on ten men, has stated that 2,000 I U of vitamin A or 4 000 I U of pure β carotene are the daily minimum for preventing impaired dark adaptation

Lewis and Haig [465] from dark adaptation studies originally put the daily minimum requirements of infants at 18–20 I.U. of vitamin A per kilogram but this was later raised to 100–200 I.U. by Lewis and Bodansky [466] who based their observations on the level of vitamin A in the blood though as this in infants has little significance (p. 25) the smaller amounts recommended by Guilbert, Howard and Hart to allow both for current needs and storage should be aimed at.

Pregnancy and lactation, which are a common cause of a deficiency of vitamin A in all countries (p. 65), increase the maternal need for vitamin A because the weight of the child both before and after childbirth should from this point of view be added to that of the mother; in fact her needs are roughly that of an 11 stone man instead of a 10 stone woman. By taking a diet rich in vitamin A she not only reduces the risk of puerperal fever (p. 37) but also increases the stores of vitamin A in the liver of the fœtus and child (p. 19), thus giving him the most important vitamin for good dental development (p. 34). It is important to give vitamin A rather than carotene during lactation because the former influences the vitamin A content of the milk far more than the latter (p. 52). The milk of well fed mothers contains ample vitamin A both for the infant's immediate needs and for increasing the low stores which are present at birth (p. 21), providing about 1,100 I.U. of vitamin A itself (p. 51). The maternal diet should be rich in fats, as there is experimental evidence that this aids the transfer of vitamin A both to the fœtus (p. 19) and to the milk (p. 52). When breast feeding is impossible cow's milk or dried full cream milks give enough vitamin A, especially if the child is given cod liver oil as well. Sweetened condensed skimmed milk containing no vitamin A is fortunately seldom bought in England, but in the tropics, where this waste product of civilization is widely sold to the natives, its consumption causes much widespread xerophthalmia and permanent blindness in children (p. 72).

It is important to build up reserves against any decrease in consumption ...

may be an inherited or familial need for unusually large amounts of vitamin A [467, 468] and at least in ...
valuable (p. 32). Adequa...
examined in England [93] ...
which the reserves are too ...
it is interesting to ponder on whether increased storage, such as apparently occurred during the war [93], indicates better or worse nutrition—an increased consumption of valuable foods or an increase in eating vegetables enforced by scarcity of meat, etc.

For workers who have to use their eyes for matching colours (p. 42) it has been found that the provision of extra vitamin A lessened eye strain and improved the health [470] so that it might even pay employers to provide free supplements of vitamin A to their workpeople. Clarkson [469] reports that in foundrymen the removal of particles of metal embedded in the cornea is made easier and fragmentation, flaking and splitting of the cornea while this is being done is prevented by the provision of extra vitamin A, and further the men report their condition immediately instead of delaying over doing so.

General physical fitness and health in normally nourished people is not improved by an increased consumption of vitamin A. Bronsby and others [340] gave one half of 1,242 children between the ages of five and fourteen a capsule of arachis oil and the other half a capsule containing 4,000 I.U. of vitamin A and 600 I.U. of vitamin D. The capsules were given every school

day for nine months *No effect was produced* on growth, nutritional status, muscular strength, the teeth, the gums or the incidence of illness In 214 *men doing very heavy manual work, neither the* weight, blood pressure, hæmoglobin, frequency of illness nor output were altered. Jenkins and Yudkin [471] gave 178 *children, aged eleven to twelve,* 5,500 I U. of vitamin A and other vitamins every school day for a year and found no alteration in the pulse rate and vital capacity or in breath-holding and the 40 mm endurance test.

In Disease. As a general rule it may be stated that most pathological conditions of the body interfere with the absorption of carotene from the bowel, and also with the absorption and metabolism of vitamin A itself Therefore in all chronic illnesses the diet should provide vitamin A itself in larger amounts than those normally required, or fish liver oil concentrates should be given.

The particular aspects of vitamin A metabolism germane to diabetes, thyroid diseases, infections, steatorrhœa, nephritis, hepatitis, pregnancy, etc, have already been described in earlier sections of this chapter

METHODS USED FOR RECOGNIZING HUMAN VITAMIN A DEFICIENCIES

Four methods have been [illegible] er a patient has a deficiency of vitamin A [illegible] *microscopy* of the conjunctiva which is [illegible] A in the blood already discussed on p 24, and measurement of dark adaptation Though the last three of these are not generally feasible, being essentially methods [illegible] nutritional surveys, this does not [illegible] ia and keratomalacia, the only [illegible] be treated without waiting for diagnosis

Clinical Examination. The clinical signs of a deficiency of vitamin A are described on p 64, so that all that need be said here is that a clinical diagnosis is only possible when the deficiency is already moderately severe, though some workers claim that a hesitant diagnosis can be confirmed by the finding of keratinized cells in the conjunctiva, respiratory tract and urine [228, 262]

Slit-Lamp Microscopy. Kruse [472] in 1941 aroused considerable interest by his claim—now known to be wholly incorrect—that biomicroscopy of the conjunctiva of adolescents and adults revealed certain subepithelial opacities which were among the earliest detectable signs of a deficiency of vitamin A Berliner [473], however pointed out that the opacities were only the common presenile or senile alterations which occur in the sub epithelial layers of the conjunctiva, and the " spots " Kruse described only the common pingueculæ It would also be most surprising that a lack of vitamin A should affect sub epithelial but not epithelial tissues Further investigations have demonstrated the frequency and variety of these conjunctival opacities both in normal adults [93, 239] and children [93, 474], and the impossibility of either causing them by vitamin A deficient diets prolonged for over two years [93] or of curing them with large doses of vitamin A given daily for two years [239].

Dark Adaptation A slight decrease in the *final* degree of dark adaptation of which the fully dark adapted eye is capable and also, though not so constantly, a slight delay in the rate of early dark adaptation are generally considered to be among the earliest signs or symptoms of a deficiency of vitamin A, though the patient himself is seldom aware of these slight disabilities

Measuring the extent and rapidity of dark adaptation has therefore been extensively used in nutritional research and surveys to unmask mild defi

ciencies of vitamin A, though it is an investigation which is not feasible in general practice since it requires both apparatus and experience. Various adaptometers have been constructed [93, 320, 475, 476, 477, 479] and the rotating hexagon, described by Livingstone [478] for rod scotometry, promises, when its uses have been more fully explored, to be of great value in investigating further aspects of the rôle of vitamin A in night vision and in diagnosing early deficiencies [93].

The papers by Harris and Abbasy [481], Yudkin, Robertson and Yudkin [477], Godding [479] and Craik [480] should be read for discussions on what instruments and what techniques give the most reliable results. The main differences in technique among different workers are the time the eyes are exposed to a preliminary bleaching of their visual purple (p. 41), the use of a fixation point during testing rather than allowing the eyes to move and so select their own most sensitive retinal area, thus avoiding any area which would give a fallacious impression of impaired adaptation due to congenital deficiency of the rods or localized pathological changes [478], though this, on the other hand, has the drawback that such instruments are less sensitive, the distance of the eyes from the test object, the size and nature of the test object, and, most important of all, whether the whole curve of dark adaptation is plotted: this is discussed later.

The broad principles of testing dark adaptation are roughly the same in all instruments. The eyes are first exposed to a bright light to cause bleaching of the visual purple after which the light is extinguished and the patient is shown small illuminated test objects. As the eyes become more adapted to the dark the illumination of the test objects can be reduced without their becoming invisible. From the different degrees of illumination of the test objects and from the time taken for the test objects to become visible with these different illuminations, a curve of dark adaptation can be constructed (Fig. 16). Children under eleven or those who are mentally dull or deaf or whose vision is bad are unfit for the test [481].

Early investigations on vitamin A deficiency were often done by measuring dark adaptation after the subjects had been only a few minutes or even seconds in the dark. But this measurement of the initial rate of adaptation may give completely fallacious results, since when the initial rate of adaptation is rapid, the final threshold or degree of adaptation may be low and it is the latter, not the former, which is *invariably* affected, if vision is affected at all, by lack of vitamin A [477]. The dark adaptation curves of two subjects may even cross as late as twenty minutes after the start of darp adaptation, so readings taken during the first twenty minutes of adaptation may give completely erroneous information about the relative state of the vitamin A nutrition of two subjects. The curves (Fig. 16) constructed by Yudkin, Robertson and Yudkin [477] clearly show the various types of curve which may be encountered, the different effects which vitamin A may have on the curves and the paramount importance, if only one reading is to be used for assessing dark adaptation, of taking the reading when dark adaptation is virtually *complete*. This in normal men occurs after thirty to forty minutes but in deficient men may even be delayed for eight hours [93]. Dow and Steven [476], Hecht and Mandelbaum [482] and Basu and De [483] have also emphasized the importance of plotting the whole curve of dark adaptation and measuring its final extent. Complete proof that impaired dark adaptation is caused by lack of vitamin A, and not by any of the other conditions mentioned below, must rest on the final threshold being raised by vitamin A. Large amounts, such as 100,000 I.U. daily for ten days or longer, should be given, and blood vitamin A estimations also carried out to make sure that the vitamin is being absorbed and is circulating in the blood [214].

The validity of the dark adaptation test for showing mild degrees of a deficiency of vitamin A is now generally acknowledged. Thus Harris and Abbasy [481] found that impaired dark adaptation was common in children

whose diet was estimated to be low in vitamin A while it was rare in well nourished children. Steel [184] found that even when using a simplified test he obtained consistent readings which, when low, were improved by vitamin A. Both these important findings have been confirmed by Yudkin *and others* [477] who in a like manner reproduced curves on different occasions for the same subject with little variation, and improved adaptation with

Dark-Adaptation Curves and the Various Effects of Vitamin A on Dark-Adaptation

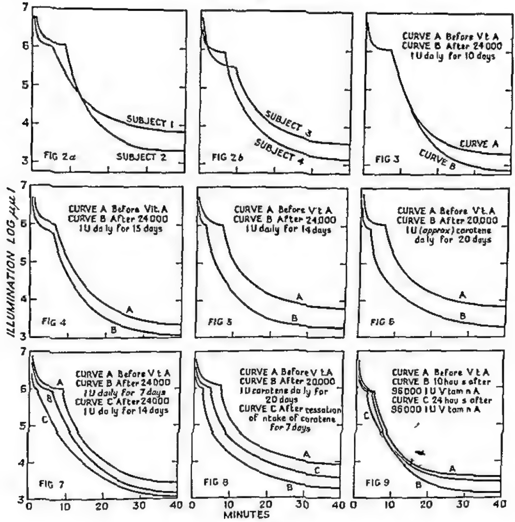


Fig. 10

Figs 2*a* and 2*b* Individual differences in curves of dark adaptation
Fig 3 Change in rod threshold only
Fig 4 Change in cone threshold and rod threshold
Fig 5 Change in rod threshold and transition time
Fig 6 Change in cone threshold rod threshold and transition time
Fig 7 Gradual improvement with continued administration of vitamin A
Fig 8 Improvement after administration of carotene relapse after cessation
Fig 9 Transitory changes produced by one large dose of vitamin A
Figs 3 9 illustrate various effects of vitamin A on dark adaptation

vitamin A. Cowell [224] reports that night blindness occurs when the level of vitamin A in the blood is below 70 I U rather than between his normal of 100 to 300 I U, and in Pett and Le Page's patients [225] dark adaptation improved as the blood vitamin A rose from 76 to 133 I U in the four hours following the consumption of a vitamin A concentrate, others have noted an improvement within one to two hours [467] ten hours [477] and two weeks [93] the dose in the last experiment being only 1,300 I U daily. In the Medical Research Council's investigation [93] dark adaptation was

impaired when blood levels fell below about 50 I U a figure congruous with the findings mentioned above since earlier techniques tended to give too high blood levels Josephs [485] also has confirmed that there is a correlation between the level of vitamin A in the blood and dark adaptation However Hecht and Mandelbaum [482] found that while dark adaptation was often promptly impaired by a diet deficient in vitamin A it took as long as six t n A

ving

I the

r here

the retina was damaged [351 478] was being stimulated [477 478] Most workers who deny like Caveness [486] the value of estimating dark adaptation have used a biophotometer the fallacies of which have been pointed out by Harris and Abbasy [481]

Robertson and Yudkin [487] have shown that dark adaptation decreases with increasing age Therefore in nutrition surveys based on dark adaptation studies allowances must be made for the age of the subjects

The season of the year has an effect on dark adaptation in men on deficient diets a slight but definite deterioration occurring in the winter [93] which is reminiscent of McCance s observations on seasonal fluctuations in the response to vitamin D (p 528)

Other changes which may be caused by lack of vitamin A are an alteration in the cone rod transition time [93 447 482] which is shown in Fig 16 and alterations in the rod visual fields which can be shown by rod scotometry [93 478]

Differential Diagnosis of Impaired Dark Adaptation It must be remembered that other conditions apart from deprivation of vitamin A may impair dark adaptation such as congenital night blindness retinitis pigmentosa or a detached retina [488] starvation with a low blood sugar [489] deficient oxygenation of the blood [478 489] lack of sleep though not physical fatigue [492] and also hysteria and possibly lack of other vitamins both of which are discussed below

The nystagmus of coal miners is not in itself a grave drawback but miners often add to it—as Culpin [490] has pointed out—functional disabilities among which may be night blindness Kellett [491] in 1939 investigated the diets of Durham coal miners and could find no proof that lack of vitamin A was the cause of nystagmus the men insisting on about 14 ounces of butter a week whatever their incomes margarine being virtually never accepted as a substitute thus whether or no the men had nystagmus they obtained roughly 1 140 I U daily from butter alone Campbell and Tonks [498] to clinch matters showed in 1948 that nystagmus is not associated with low levels of vitamin A in the blood though they reported that miners have a raised threshold for dark adaptation which is not due to lack of vitamin A Campbell [495] in 1941 reviewed most of the relevant literature

A further type of purely functional night blindness may develop among soldiers who have to carry out dangerous duties in the dark This was recognized in England in 1917 but was not publicly reported at the time because it was commoner in the German than English armies leading to wastage of men in the former where its cause was not understood [490] In 1941 Wittkower and his collaborators [496] examined fifty two soldiers complaining of night blindness Most were found to have severe psychological disorders not in origin due to fear of fighting The surprising and to us unjustifiable conclusion drawn from this examination is that most cases of night blindness seen in this country are probably of psychological origin It seems more probable that only the psychopath and malingerer spontaneously complain of night blindness *normal people are seldom worried* by the condition Harman [497] has discussed how to recognize the malingerer

The importance of vitamins other than vitamin A for dark adaptation

is uncertain, but the work of Harris and Abbasy [481], of Stewart [492], of ... is important This is ... def ... of six subjects with p... dark adaptation ... ndness and a deficien... of ascorbic acid, and five subjects with low normal adaptation. Nicotinic ac... though an essential ingredient of the visual purple cycle (p. 41), has not be... reported to be of clinical importance in dark adaptation. A paper by Kim... and Gordon [493] stresses as well the value of riboflavine. This latter pa... has been frequently quoted apparently without having been read, becaus... is generally believed to state that riboflavine is in some cases necessary for t... formation of visual purple ... in a few patients who had ... A, large doses of vitamin A ... when both became normal In other words ... for the absorption or mobilization of vitamin ... once there was an adequate supply of vitamin A in the blood the eye ... could not utilize it normally ... and well controlled complet... that riboflavine alone does ... has not yet investigated if this is due to a direct action on ... an indirect action through raising the level of vitamin A in the bloo... Morton [504] in 1944 reviewed the evidence in favour of riboflavine and n... vitamin A being the chromophoric group of visual purple

Having excluded all the above factors which, apart from lack of vit... ... there still remain various physiolog... ... shown will produce individual diffe... ... l in the light retards dark adaptatio... astens it. Dark adaptation is always slow... the pupil, and, though general colouring h... no effect, heavy pigment... of the retina favours more rapid adaptatio...

Alcohol and amphetamine may, according to Yudkin [500], improve da... adaptation even when they cause no increase in the blood vitamin A Th... improvement is transitory, is beyond that obtainable with vitamin A, an... is apparently not related to the mental effect of the drugs No such effe... was obtained by the Medical Research Council [93] with alcohol, though ... and maybe other drugs probably raise the level of vitamin A in the bloo... (p 28) Only a few negative observations have been made on the effe... on dark adaptation of thyroid, dinitrophenol, caffeine, bromide, strychnin... phenobarbitone and morphia [247, 500, 502, 503]

HUMAN DISEASES DUE TO A DEFICIENCY OF VITAMIN A

M... people in England and America take so little carotene or vitamin A ... of a deficiency, or are so close to ... periods when vegetables, ... when the amounts of these which can be eaten or bough... rationing, old age, ill health or unemployment. Figures based on what percentage of the population has poor dark adaptation or skin changes give too rosy a picture of their nutritional condition, as a recognizable deficiency only occurs when the body has already broken down from lack of the vitamin, this breakdown being preceded by a period when the functions of the body are at best being carried on under an extra strain

Changes in the skin and poor dark adaptation are the earliest signs of a definite deficiency which can be diagnosed Judging by poor dark adaptation, more than half the children from working class homes in England were found to be deficient by Harris and Abbasy [481] in 1939, while America in 1940 had a similar or even worse percentage [467] In some parts of the

tropics a deficiency must be almost universal since Fitzgerald Moore [441] reports that in the Philippines nearly one third of the children attending a hospital before the war had xerophthalmia and this has become even worse since the Japanese invasion [505], while in the Dutch East Indies with a child population of half a million, nearly four thousand children between the ages of twenty one months and fifteen years were blind apparently from lack of vitamin A [441] Both in Africa and India skin changes due to lack of vitamin A were present in eighty per cent of groups of children [507]

English adults a deficiency is apparently common than in n Harris and [481] in 1939 only slightly d dark adapta half of thirty king class in three of middle class ugh in 1948 e fifth of uni udents in Lon affected [512] merica the ar to be one third of and sixty students und to be

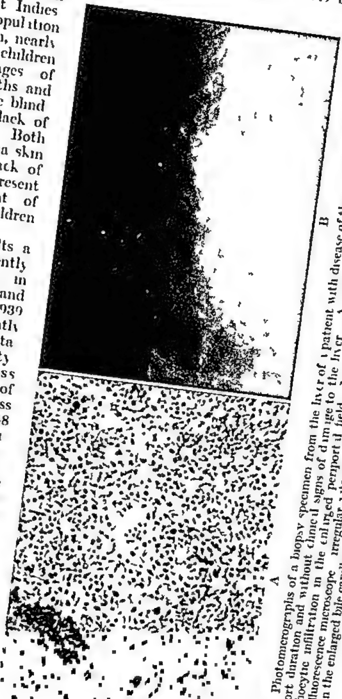


FIG 17 Photomicrographs of a biopsy specimen from the liver of a patient with disease of the gallbladder with jaundice of short duration and without clinical signs of damage to the liver A routine histologic examination lymphocytic infiltration in the enlarged periportal field No visual damage to the parenchyma B examination with fluorescence microscope irregular vitamin A fluorescence is imparted almost only by Kupffer cells The bile casts in the enlarged bile capillaries appear black (*See also* Figs 3 and 5)

[224 484]" Holland [509] and 0]

of lack of vitamin A on (*a*) the skin (*b*) the eyes (*c*) the and (*d*) other tissues will be considered separately

f Lack of Vitamin A on the Skin (*Toad Skin or Phrynoderma, ratosis Pilaris Icthyosis Follicularis Lichen Pilaris, Lichen ner's Disease, etc*) In England skin changes due to l

vitamin A probably do not occur or occur only as an extreme rarity. folliculosis or follicular keratosis (Figs 18 and 22) in children ascribed lack of vitamin A by Pemberton [515] and other observers during the l thirties and early forties is now known to be a normal skin condition fou in up to eighty per cent of children Stannus [513] from his very extens experience gained in nutrition surveys of English children during the w has described the condition in detail and reviewed the evidence about cause It appears to be the customary reaction of the skin to exposure to weather, to the trauma of clothes and to dirt, being most pronounced on fronts of the thighs above the knees where the bare skin is chafed but protected by "shorts" and skirts Unlike the true toad skin caused l deficiency of vitamin A, it is common before puberty and increasingly later and, moreover, its papules never grow so large nor spread so wi

The skin changes which do occur as a result of lack of vitamin A w described by Nicholls [507] in Indi 1933, who first used the name toad or phrynoderma, and by Lowenthal [5 in the same year in Africa, who made observations without knowing that importance of vitamin A for the skin already been recognized two years bef in China by Frazier and Hu [30 Pallister [514] in 1940 found toad s common in Malaya.

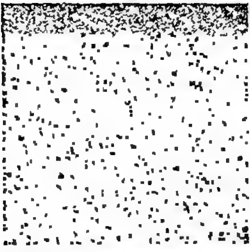


Fig 18 Life size skin print showing mild folliculosis on the front of the thigh of an English schoolgirl Skin prints, in contrast to photographs, enable records of the condition of the skin to be made rapidly, easily and cheaply Prints should be viewed

There is, however, still some unc tainty as to whether toad skin is to a simple deficiency of vitamin A whether some other factors are a involved. In favour of a deficiency vitamin A being the only cause is t work of Lowenthal [511] who cured t of his cases with vitamin A alone, a practically all the rest with cod-liver while Lehman and Rapaport [467] cur their cases with halibut-liver oil *Pallis* [514] in *Malaya noted an associati* between toad skin and Bitot's spo Steffens and others [516] produced typi changes in the skin of a man by a vitam A deficient diet, and Nichols [507] a Lowenthal [511] have noted a cl association between skin changes and night blindness or xerophthalmia, th association being apparently far commoner after than before adolescence

Against these observations, however, must be put those of Aykroyd an Rajagopal [517] and Rao [521] who did not find any close correlation betwe toad skin and xerophthalmia, or between the former and a diet deficient vitamin A during a very extensive investigation of Indian schoolchildre Frazier and Hu [303] and Sweet and K'Ang [262] found no correlation all between the condition of the skin and eyes, so that they decided that children the eyes but not the skin were affected by a deficiency of vitamin while after adolescence the skin chiefly suffered This is confirmed by Frazi Hu and Chu [304] who showed that in young children the skin is general only xerotic and atrophic, follicular hyperkeratosis *seldom occurring* befo adolescence (Fig 70) This has been the experience of many other worke [505, 545 546] The problem is still further complicated by the descriptio given by Fox [519] and Wiltshire [518] of the early skin changes in scurv (p 70) which appear to be almost identical with those of toad skin Th position appears to be that lack of vitamin A alone can cause toad skin, b

that there is often some other factor which alters the reaction of the skin so that it is more sensitive to a deficiency of vitamin A. This ancillary factor may be either a second food deficiency [521] or the stage of sexual development [304] or a familial need for abnormally large amounts of vitamin A [467, 515] or a racial susceptibility such as is apparently shown in India [507, 517] and Africa [506] but not in China [262, 303, 304]. Whatever the cause the reaction of the skin varies so much that sometimes changes occur before there

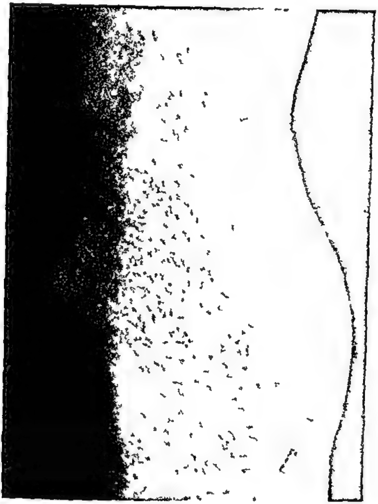


FIG. 19. Diffuse involvement of hair follicles due to vitamin A deficiency in a fourteen year old Chinese boy. His skin is also shown in Figs. 20 and 21. Hyperpigmentation of the conjunctiva was the only ocular sign of vitamin A deficiency.

is any obvious involvement of the eye [303, 304, 508, 517] or even slight impairment of dark adaptation [516] while in other cases the eyes may be seriously damaged while the skin apparently remains normal.

The insidious onset of a dry, rough skin, especially in those areas where the papular eruption occurs later, is the first cutaneous symptom of a deficiency of vitamin A. Loewenthal [506] and Frazier and Hu [303] stress this early symptom which has been noted at all ages from infancy to old age and in both sexes. There is an [illegible] the deficient winter diet. The dry skin [illegible] al eruption which often spreads rapidly over the fronts and sides of the thighs and the

FIG 20 Follicular hyperkeratosis, with projecting horny spines, before treatment (See also Figs 19 and 21)

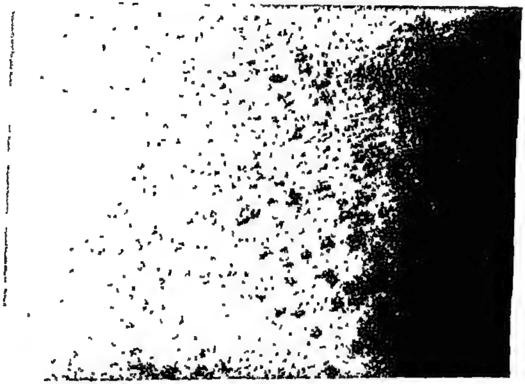


FIG 21 The same case shown in Figs 19 and 20 after five weeks' treatment with a vitamin A concentrate and cod liver oil which provided about 63 000 I U daily

posterior and lateral sides of the forearms just below the elbows, and the fronts of the arms and shoulders. Some observers report that the eruption generally spares the front of the chest [507], the groins and axillæ, and the backs of the hands and feet [303, 304], but others state that it may ultimately cover the whole body apart from the face, which is seldom involved [507, 521] though "black-heads" are common [262, 303, 304] and may in Europe be the dominant symptom. The scalp is not affected, but the hair may be dry and brittle and the nails may have transverse or longitudinal ridges [262], though generally the hair and nails are normal [303, 304] Increased pigmentation [262] both of the papules and the skin, which has been likened to argyrosis by Mu and others [262], is sometimes seen in coloured patients,

FIG 22. Follicular hyperkeratosis in a boy aged fourteen (Great Britain) Note the plugging of the hair follicle with keratinized material, the absence of the sebaceous gland, the increased keratinization of the superficial layers of the skin, and the infiltration of small round cells near the base of the follicle

being analogous to the scleral pigmentation (p 74) Itching has been reported to be present [507, 511] and absent [521]

The eruption consists of dry horny round or oval sharply defined papules, varying in diameter from that of a pin's head to as much as a quarter of an inch [511] The size of the papules increases with the duration of the deficiency in the early stages being more easily felt with the fingers than seen, while later the skin looks from a distance as if many split lentils had been stuck upon it Each papule is formed by hyperkeratosis of the pilosebaceous follicles, and has a hard keratinous core which can be picked out, leaving a small pit Often broken or coiled up unerupted hairs are found either projecting through the papule or imprisoned beneath. The papules seldom, if ever, undergo pustulation [262, 507, 511, 521], though Young [523] believes that the skin is more susceptible to fungus infections. Rao [521] and Frazier and others [303, 304] have reported skin changes in keratomalacia which are typical histologically of phrynoderma apart from the papules (Fig 70)

Microscopical examination of the skin in phrynoderma shows that the papules are composed of masses of keratinized cells which have been shed to

the pilosebaceous follicles becoming compressed in their centres into horny amorphous plugs [511, 521]. These block the hair follicles and the sebaceous glands which then tend to atrophy. There is also hyperkeratinization of the epidermis especially round the papules, and a thickening of the stratum corneum and sometimes an increase in the pigment cells and a mild lymphocytic infiltration near the base of the follicle. The sweat glands are not markedly changed but do not appear to be secreting and they are often plugged with keratinous material. Vitamin A, in spite of its importance for the skin, is not present in the epidermis [58].

The differential diagnosis is *from acne and from early scurvy*. In acne the skin is greasy instead of dry, the eruption is mostly limited to the face and front and back of the chest, unlike that of toad skin, and it mainly occurs during adolescence and early adult life. Pustulation of the papules is the

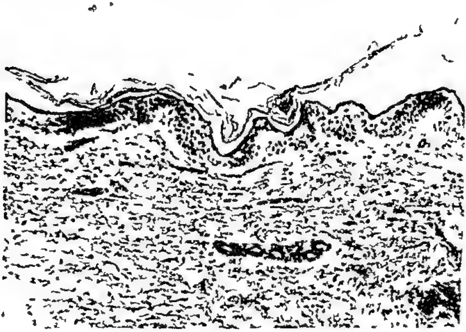


FIG. 21. Showing the atrophic condition of the epidermis of a Chinese child approximately three years of age who had generalized xerosis of the skin but no obvious follicular keratosis. There was pronounced xerosis of the conjunctiva, which was wrinkled and leathery, and early keratomalacia. The child had had diarrhoea for a month. The section is from the abdominal wall.

rule rather than the rare exception, generally leaving behind small scars which are never seen when uncomplicated cases of toad skin are cured.

The earliest sign of scurvy, according to Wiltshire [518] and Fox [519], is a skin eruption which is identical with that caused by lack of vitamin A, since the skin is dry and the papules are formed in the mouths of the pilosebaceous follicles by masses of keratinized epithelium in which or under which are broken or coiled up hairs. But these papules appear to be rubbed off easily and leave behind them pink follicles which have not been described in toad skin, and also their distribution is slightly different, being more confined to the legs. The perifollicular hæmorrhages which would confirm a diagnosis of scurvy only appear some weeks after the papules.

Chronic ulcers of the skin are a very rare complication of phrynoderma and when they do occur only heal with vitamin A [262, 524].

Treatment with vitamin A is entirely successful. The first sign of recovery is a return of sweating so that the skin within two or three weeks no longer

feels dry [303 304 467] though it does not return to normal for two to nine months the shorter period being on very high doses of vitamin A such as 100 000 I U a day Concentrated preparations or even injections should be used when diarrhœa is present [262] or aqueous dispersions (p 18) should be of value The keratotic plugs in the follicles are reported to be extruded as tiny rice like bodies but by remaining partly adherent to the skin they give to it a shaggy appearance [467] Ultimately the epidermis and the follicles return to normal and new hairs develop

Darier s Disease Since Peel and his collaborators [527] in 1941 suggested that Darier s disease was due to a deficiency of vitamin A many further papers have appeared on the subject [528 529 530 534 536 537 538] A few cases respond to large doses of vitamin A more do not Therefore in so intractable a disease it is reasonable to try vitamin A though most unwise to promise any improvement Either of the two genetic types of the disease may respond Carleton and Steven [529] curing a mother but not her son and a brother but not his sister while Lissia [537] failed to cure three familial and one idiopathic case The level of vitamin A in the blood is just within normal limits [529 534 536] or very low [527 536] and is raised and maintained in most cases only by continuous and large doses of the vitamin [527 536] though even these may fail to cause a rise [536] Hepatic function is often impaired [536] as may be dark adaptation [527] though both may be normal [529 536] The doses of vitamin A generally given by mouth have ranged from 100 000 to 300 000 I U daily and should be continued for at least two months before abandoning them as useless Occasionally large doses cause an exacerbation the papules becoming bullous [528]

FIG 24 Keratomalacia in a Danish infant (see p 2) The disease arose after two months feeding with oatmeal After treatment with cod liver oil the right eye improved and became almost normal The left cornea is largely necrosed

Tylosis or Hyperkeratosis Congenitalis Palmaris et Plantaris or Mal de Meleda Brunner and Fuhrman [539] successfully treated one patient with this rare and reputedly incurable hereditary condition giving oral daily doses of 300 000 of vitamin A When treatment was stopped there was a relapse followed again by recovery when treatment was restarted Smaller doses were less effective Porter and Haber [540] cured the keratosis on the palms of their patient and improved the soles by daily doses of 100 000 to 200 000 I U for six months the palms were cured after three months and the improvement persisted six months after treatment had stopped Porter [605] later reported improvement in three of six cases

Ichthyosis Rapaport and others [531] in 1942 reported six cases of ichthyosis all of which were very considerably improved by vitamin A and also gave references to similar results obtained by two other workers The vitamin was given orally in daily doses of 60 000 to 200 000 I U or intramuscularly two or three times a week in doses of 100 000 I U Some cases responded only to the former method of administration and some only to the latter Improvement occurred within a few weeks but relapses especially in the winter, were common if the treatment was stopped All five cases who were tested had poor dark adaptation and in five cases there was a family history of ichthyosis The level of vitamin A in the blood is low [538] Porter [605] reports improvement in one case out of nine

Eczema The absorption of vitamin A in infantile eczema is said to be impaired [532] and the level in the blood low [167] Gross [533] reports curing eighteen of twenty four cases of nummular eczema with 75 000 U S P units daily Leitner and Moore [538] found the average level of vitamin A in the blood of twenty six adult patients was higher than in control patients (See also vitamin F, p 677)

Other Skin Conditions Many other skin conditions have been treated by vitamin A but the review by Carleton and Steven [529] and investigations on the level of vitamin A in the blood of patients with thirty two different skin conditions [534 538] leave little doubt that vitamin A will benefit none other than those already discussed with the possible exception—when given in doses of 100 000 to 500 000 I U daily—of *pityriasis rubra pilaris* [535 541] *pachyonychia* [540 605] *acne vulgaris* [542 543] *senile keratoses* [543] and *lichen ruber planus* and *psoriasis* [544]

The Effect of Lack of Vitamin A on the Eyes The earliest detectable result of a lack of vitamin A on the eyes is a slight impairment of dark adaptation the underlying physiology of which has been described on p 40 and the methods for its detection and its differential diagnosis on p 60 so that here it is only necessary to point out that the condition is seldom noticed especially by those living in well lit towns until it has become very pronounced Few patients with mild night blindness are sufficiently observant to realize that their twilight vision is better in the early morning than in the evening though this valuable diagnostic point was noticed years ago in badly fed slaves [488] Sometimes however as in poorly fed Newfoundland fishermen [1] night blindness comes on suddenly after a long day in very bright sunlight so that there is complete blindness in the evening dusk though normal people are seeing perfectly Sudden changes in diet R ins during the Lenten fast may also precipitate

are far more liable than are older children and adults to keratomalacia and permanent blindness due to lack of vitamin A The first change in the eye which can be observed clinically is a drying or xerosis of the eye known as xerophthalmia which is generally accompanied by photophobia In early cases the condition may be unmasked by holding the eye open for a minute or two when the lustre is lost through the rapid drying of the eye This is due to the metaplasia of the conjunctival epithelium stopping the secretion of the mucous cells which can be confirmed by finding keratinous cells in gentle scrapings of the eye [262] Wrinkling of the conjunctiva can also be seen at an early stage Later the conjunctiva may become thick and leathery from the gross keratinization (The diagnostic value of slit lamp examinations of the conjunctiva is discussed on p 60)

Once xerosis is present no further changes may occur or the whole eye may be rapidly destroyed The patient suddenly complains of a feeling of a grain of sand in the eye which is followed by photophobia lacrymation inflammation and a sticky discharge These symptoms are due to the dry thickened conjunctiva wrinkling up which gives the sensation of a foreign body in the eye, while the discharge is caused by a secondary infection [262]

The condition is now serious since the stroma of the cornea may become œdematous necrotic and weak so that keratomalacia occurs The first sign is a spreading opaque white spot in the cornea which can grow so rapidly that the sight may be lost in a few hours At the same time secondary infection may lead to ulceration and finally perforation of the weakened cornea with destruction of the eye This fulminating course is commonest in infants [468] even having been present in one of Maxwell s [314] at birth but it can occur at any age [279 280]

One quarter of Sweet and K Ang s cases [262] and of Blegvad s [168] had one eye involved for several weeks before the other

The vascular changes which occur in the cornea and their possible relation to a local secondary deficiency of riboflavine are discussed on p 34

De Haas and Meulemans [510] investigating vitamin A in the blood of children suffering from xerophthalmia found none in six and 4 6 and 8 I U per 100 c c in three and 22 I U per 100 c c in one with perforation of the cornea

Recovery apart from scarring of the cornea is always possible if perforation has not taken place The treatment of acute cases in infancy is to give urgently vitamin A Aqueous suspensions (p 18) when available should be used a first dose of 100 000 I U being followed daily by a 10 000 I U for week after which cod liver oil in normal amounts of about two teaspoons should be sufficient Four to six times these quantities are needed by older children and adults When only cod liver oil is available as much must be given as the patient can tolerate while highly potent fish liver oils should be used in doses about fifty per cent higher than those given in aqueous suspensions Injections act too slowly (p 27) to be as valuable as oral treatment

FIG 25 Bitot s spots in a Singhalese child The left eye shows thickening pigmentation and white striated patches of the temporal bulbar conjunctiva This was also present in the right eye The nasal bulbar conjunctiva was free from these changes

Local treatment of the eye should be confined to saline irrigations when there is discharge given with great gentleness by the doctor himself or a nurse of experience since the weakened cornea may easily be ruptured [546] Sulphonamides and antibiotics are not necessary nor are local applications of cod liver oil of value since Rotth [189] found that in rats with xerophthalmia cod liver oil placed only in one eye cured the other just as quickly while the general condition of the animals improved which shows that the effect of vitamin A was not local but general through its absorption into the body

In infants [468] bronchopneumonia due to infection of the metaplastic epithelium of the respiratory tract is the usual cause of death At all ages [280 505 545 546] gastro intestinal disorders and intestinal worms being both a cause and consequence of lack of vitamin A often precede or follow xerophthalmia

Prolonged mild deficiencies of vitamin A lead to the formation of Bitot s spots which were first described by Bitot [549] in 1863 in patients in the foundling hospital in Bordeaux *Un assemblage de points d un blanc eclatant produisant comme une tache nacree ou argentees a cote de la cornee transparente* The taches fluctuated in size with the degree of night blindness being larger when it was most severe They were always placed just lateral to the cornea on the equator of the eye generally being in the shape of a triangle whose base was slightly concave and about 5 mm in

width while the sides of the triangle were about 8 mm Sometimes however, they were round or oval and might also be composed of fine lines as well as dots Very occasionally a few scattered dots were seen to the inner side of the cornea Bitot also pointed out that the ' taches were a definite change in the conjunctival epithelium itself which might have either a rough or striated surface Nicholls and Nimalasuriya [550] from extensive observations on several hundred cases in children in Ceylon have confirmed this picture though night blindness was not usually present They state that the first changes are a slight thickening and pigmentation of the scleral conjunctiva which is followed by a heaping up of epithelial cells which stand out white against the pigmented background and are generally striated looking like a dab of chalk paste striated with a pin ' They are only seen on the inner side of the cornea in two per cent of cases, they never ulcerate never occur over the cornea itself though generally approaching to within 1 or 2 mm of its edge and mostly appear in only one eye May and Wolffe [526] reported that in their English infant the triangular Bitot s spots were on each side of the cornea and appeared to be covered with foam and Aykroyd and Rajagopal [517] describe the Bitot s spots in their Indian children as yellowish foaming patches Nicholls [550] suggests that this foamy appearance is due to a very rapid and loose piling up of epithelial cells which does not occur in communities where a chronic deficiency of vitamin A for generations has led to a more chronic and slow reaction to the deficiency The increased general pigmentation of the sclera—also noted by Mu [520]—probably only occurs in dark races and its diagnostic value before Bitot s spots appear is still uncertain Early conjunctival changes only visible with a slit lamp are discussed on p 60

Treatment is with large doses of cod liver oil or vitamin A concentrates recovery generally being slow [517 550] as it is in phrynoderma though it may only take two weeks in young children [505]

Blumenthal [554] describes a very peculiar keratitis which he has observed frequently in the South African Bantu He ascribes it to malnutrition and especially to lack of the vitamin B complex In the uncomplicated form in children the cornea dissolves away quietly and insidiously at one small point followed by a prolapse of a knuckle of iris without pain discomfort inflammation or infection In adults the central or whole cornea may soften and expand with or without compensatory thickening and rupture Recovery especially in children is dramatic and complete when mixed vitamins are given

Asthenopia is stated to be often caused by lack of vitamin A patients continuing in spite of the usual treatments to complain of a dislike of bright lights headaches and difficulty in seeing while driving or at theatres or fatigue whenever they use their eyes Impaired dark adaptation is present Cordes and Harrington [551] gave 30 000 I U of carotene daily for a month with complete relief of all symptoms in seventy nine per cent of eighty two cases between the ages of thirteen and seventy three though as some of their patients became yellow during the treatment vitamin A should be used if such large doses are really necessary Similar results have been reported by Vanzant [552] and we have found 48 000 I U of vitamin A daily very effective Follicular conjunctivitis in children has been stated to be due to lack of vitamin A and to respond rapidly to 13 000 I U daily [553]

The Effect of Lack of Vitamin A on the Nervous System Lathyrism

The experimental work discussed on p 42 shows that deprivation of vitamin A causes degeneration of the nervous system in animals though the degeneration is surprisingly severe before there are any clinical symptoms In man no neurological symptoms are generally associated with those conditions such as keratomalacia where they would be expected if the central nervous system were affected by lack of vitamin A There is however the possibility that in man as in animals the degeneration gives such tardy symptoms

that they seldom occur before the deficiency has been remedied or death occurs unless the degeneration is accompanied by a second deficiency (p 43) This is supported by Nicholls [505] who in Ceylon constantly found degeneration of the spinal cords of children dying with symptoms of a vitamin A deficiency clinically deficient ften have transitory i te to lack of vitamin A since this is very common while beri beri is rare He previously reported [507] that prisoners with phrynoderma and eye signs generally had neuritis and diarrhœa the latter condition may have caused or increased a deficiency of vitamin B_1 or both may have been further symptoms of lack of vitamin A (p 77) In the same way as phrynoderma may require both lack of vitamin A and some other factor for its development (p 67) Mellanby [361] from his work on puppies has suggested that vitamin A is necessary for the health of the nervous system in man its lack however only becoming important when a secondary deficiency or some toxin is present so that beri beri is accentuated by lack of vitamin A and gangrene in ergotism occurs alone when vitamin A is plentiful but when it is not convulsions also appear because the nervous system needs vitamin A to protect it against the ergot Mellanby also suggested that lathyrism is a disease not only due to toxins which are always present in the germ of grain but also to lack of vitamin A since puppies on a high cereal vitamin A deficient diet developed a more serious degeneration of the cord when they were also given rye germ and in two cases wheat germ or dried beans

Lathyrism is a disease which in the past has been ascribed to the eating of a vetch of the *Lathyrus* family during periods of famine either deliberately in areas where it is used as cattle food or by mistake where it has grown among the corn But the published accounts of the disease give such varying symptoms and in several instances so definitely rule out the possibility of any kind of *Lathyrus* being the cause that it appears certain that lathyrism is not one disease but a group of rather similar diseases only sharing in common a background of famine and some form of paralysis of the legs

The only cause common to all outbreaks of lathyrism is a deficiency of food which apparently weakens the resistance of the lower segments of the spinal cord to various toxic agents though with proper nutrition these would be harmless The particular symptoms of each outbreak depend on what particular toxic agent is present these have been extensively studied while some work has also been done on what particular ingredient of the diet is deficient Considering the latter first the importance of vitamin A is stressed by Young [557] who not only found night blindness common in a village suffering from lathyrism but also noticed that the disease did not occur in neighbouring villages where the diet contained as much *Lathyrus* but more vitamin A fish and meat while Shah [558] has reported great improvement in patients when vitamins A and D were given Apart from night blindness no deficiency diseases have been reported as occurring with outbreaks of lathyrism so that it seems improbable that lack of any vitamin apart from vitamin A is a factor The nervous degenerations caused by vitamin E (p 647) and those due to lack of some unknown factor reported by Wintrobe and others [359] do not it is true give any except neurological symptoms but they are in essence progressive while lathyrism is a disease which never progresses beyond the initial paralysis But of course lack of other substances in the diet apart from vitamins may be important which is suggested by Basu and others [559] who found that the seeds of *Lathyrus sativus* which often form the staple food in famine villages are a very poor source of protein being especially deficient in tryptophane Minchin [560] believes that some protein deficiency may be the important factor while McCarrison s experimental work with pigeons [561] on the effect of manurial conditions on the nutritive value of millet and wheat suggests that in areas

where husbandry is poor grain may not only be less nutritious but even toxic

Of the factors which actually injure the already debilitated nervous system and so are the immediate precipitating cause of lathyrism some toxic substance in vetches of the *Lathyrus* family has for long been postulated As early as 1770 an epidemic in France causing paralysis of the legs was thought to be caused by eating vetches and a similar outbreak was seen in England in 1785 while in 1840 *Lathyrus cicera* was reported to cause paralysis when fed to rabbits [562] In 1882 *Lathyrus cicera* or *clymenum* was held responsible for an outbreak in Syria which affected 1 200 people [563] and in the long French review of recent lathyrism in Syria *Lathyrus sativus* is held by Trabaud and his colleagues [563] to be the cause of lathyrism even when only a few seeds have been eaten But they found this vetch harmless for the lower apes camels cows fowls rabbits and dogs as did Basu [559] and others [564] for rats and Anderson [565] for monkeys and other animals Lewis and others [567] investigated eight species of *Lathyrus* some were toxic for rats and mice but a disease similar to lathyrism was not produced None of these experiments however are really conclusive since the animals were not on a famine diet analogous to the diets taken by men prior to develop ing lathyrism nor was the vetch grown in the same soil as that around villages where lathyrism occurs On the other hand Anderson and others [565] showed that the seeds of a weed *Vicia sativa* and also an alkaloid extracted from them affected the nervous system of monkeys and other animals while Shah [558] investigating an outbreak of lathyrism found that seeds of *Vicia sativa* but not of *Lathyrus* had been eaten mixed with the corn Young [557] however reports that his cases had eaten *Lathyrus* but little or no *Vicia sativa* and Minchin [550] describes lathyrism without *Lathyrus* as does Gopalan [566] while Spillane [568] saw a condition like lathyrism in Japanese prisoner of war camps where there was starvation but no *Lathyrus* had been eaten

To sum up it seems that lathyrism is due to lack of vitamin A and a poor diet paving the way for toxic agents to attack the nervous system these toxic agents not always being the same and therefore not always causing identical damage and symptoms Probably they are sometimes alkaloids found in the seeds of *Vicia sativa* sometimes alkaloids present in the seeds of various kinds of *Lathyrus* grown in particular soils sometimes no clue is given as to their nature so that one is forced to consider whether lathyrism may be due to an infection by some organism which is only pathogenic when the diet is impoverished

Men are generally reported to be affected far more frequently than women [557 560 564] though not always [563] and the condition occurs at any age [557 563] though mothers do not transmit the disease by suckling their children [563]

The clinical picture of lathyrism varies in every outbreak Minchin [560] whose patients had eaten no *Lathyrus* found that the onset of the paralysis of the legs may be sudden or slow McCarrison [564] says the incubation period on bread containing *Lathyrus* is two to six months while Trabaud [563] states that four days to four weeks after eating *Lathyrus* a tingling starts in the legs which progresses to a tremor that is present at rest but changes to a spastic stiffness on walking The patients may never be ill in themselves [560 563] but Young [557] noted that there is a previous fever and Shah [558] that the condition begins with gastro intestinal symptoms

Shah [558] reports that his cases had both spastic paralysis of the legs and sensory impairment and that occasionally the arms were involved which also occurred in a few cases of Minchin s [560] but all other reports emphasize that there is a pure upper motor neurone involvement of the legs alone so that the patients walk as if balancing along a rail [557] without any loss of their sense of position The condition of the reflexes is very puzzling s n

Minchin [560] observed that while the legs were spastic with extensor plantar responses, the cremasteric and abdominal reflexes remained normal, even in some cases where the arms were affected. Trabaud [563] found completely normal reflexes, including the plantar responses, though there was spasticity and clonus of the legs. The cerebrospinal fluid in new cases gives a paretic curve [560] and in old cases is normal [563].

Trabaud suggests that the damage is localized in the pyramidal tracts to the lower limbs because of changes in chronaxie. Minchin [560] noted that the bladder was occasionally affected, but other observers have not reported this.

Sexual impairment has been noted by earlier writers, but it is not mentioned in recent English reports from India, while the French observers [563] point out that lathyrism is an ideal disease for the Eastern male, since while it prevents him from working it in no way hinders him from enjoying those pleasures which are necessary for continuing his family tree. Neither pregnancy nor lactation are affected [563]

The disease is never progressive after a few days or weeks, and is generally

FIG 26 Trachea of an American infant showing stratified keratinizing epithelium

considered incurable, though Shah [558] reports great improvement with cod-liver oil

Other Effects of Lack of Vitamin A. Frequent respiratory infections [262, 468] and diarrhœa [262, 280, 468, 505, 524, 545, 546] are the only symptoms, apart from those of the eyes and skin, which are frequently reported as occurring with a deficiency of vitamin A Neither of these are of much value in diagnosis, though the cough is typical, being unproductive because of the blocking of the mucous glands by desquamated epithelia, and its cause can sometimes be confirmed by examination of nasal scrapings for metaplastic changes in the cells [262] Children with xerophthalmia usually die of bronchopneumonia [468] The diarrhœa has been cured by Pillat [524] and Sweet and K'Ang [262] with fats and vitamin A

Children with mild chronic deficiencies are lively and active [549, 550] and have been described as podgy [524], but they are small for their age and have a high death-rate [550]

Post-mort

is a

lacl

either the skin (p. 65) or the eyes (p. 72) being damaged separately. This means that the diagnosis of a deficiency need not be discarded because all

the typical changes are not present Sweet and K Ang [262] have carried out the largest number of post mortem examinations of patients dying with a definite deficiency of vitamin A Of seventeen cases eight had metaplasia of the epithelium of the larynx or trachea which sometimes even involved the small bronchi and the mucous glands whose ducts were blocked by the desquamated epithelium In five cases the epithelium of the œsophagus was affected though the rest of the digestive tract was normal apart from the pancreatic ducts in one case Changes were observed in the renal pelves of three cases but the ureters and bladders were normal and so were the prostates except one of ten males Of seven females there were changes in the uterine mucosa of one Hæmosiderosis was present in the liver and spleen of half the cases but this was not regarded as being due to lack of vitamin A In Wilson and Du Bois child [273] the respiratory tract was extensively involved and so was the pancreas the ducts being blocked by the shed cells and the acini cystic The islets of Langerhans were normal but in the thymus Hassall s corpuscles were enlarged There was metaplasia of the renal pelvis which was also noted in Boyle s infant [293] together with tracheal changes and defective tooth formation

The relationship of vitamin A to other diseases and the part which it plays in the normal physiological function of the various systems of the body have been discussed in the earlier sections of this chapter

CAROTINÆMIA, XANTHOSIS CUTIS AND HYPERVITAMINOSIS A

Carotinæmia This is a word which is loosely used to mean excess of carotene in the blood though what constitutes an excess is unknown Normal values are roughly 50 to 240 micrograms per 100 ml depending on the diet [569] When the carotene rises beyond a certain level in the blood which varies with the individual xanthosis cutis or yellowness of the skin occurs and as this is the symptom which draws attention to the excess of carotene in the blood carotinæmia is increasingly used as a synonym for xanthosis cutis This is incorrect as many patients with lipoid nephrosis or nephritis have a carotinæmia which does not discolour their skin though it would do so in normal people The explanation of this is obscure it may be due to the glands of the skin failing to secrete carotene owing to their dysfunction caused by the nephritis or to the carotene being anchored in the abnormal nephritic blood

Carotinæmia without xanthosis cutis is probably a harmless condition though Clausen [97] in a very thorough investigation showed that both very high and very low levels of carotene in the blood appeared to decrease slightly the resistance to respiratory infections In animals Davies and Moore [123] could not produce a toxic state by giving huge amounts of carotene although Sherwood and others [389] report that doses of carotene equal to about 1 500 I U of vitamin A stop œstrus and libido in rats

Xanthosis Cutis Stannus [409] in 1929 gave an extremely valuable review of clinical reports about this condition up to that date while Joseph s paper [570] in 1944 should be read for its bibliography and summary of later clinical and experimental work

The causes of the high level of carotene in the blood which leads to xanthosis cutis are pathological or dietetic the former which are mostly discussed elsewhere include metabolic diseases such as myxœdema (p 50) tuberculosis [409] disturbances in lipoid metabolism (p 50) and possibly failure to oxidize carotene [409 570] The dietetic cause of xanthosis cutis is simply the unduly high consumption of vegetables containing carotene such as happened in England in 1942 when the Ministry of Food owing to a glut of carrots advertised their virtues in every newspaper and even com missioned Walt Disney to draw the carrot family In 1943 the carrot fly ruined the crop and propaganda and xanthosis cutis both declined

The amount of carotene in the diet which causes xanth
with the individual Two to three pounds of raw carrots w
eaten by Thomson's patient [574] for eleven months while
pounds weekly for seven months was the smallest quantity w
consumed by Almond and Logan's five patients [573], thus
carotene equivalent in spinach had no effect on the colour of
roughly two and a half months in Hoch's three experimental
though double this amount did so within nineteen and twenty
subjects Hoch noticed that the steady uniform rise in the bl

FIG 27 Swellings in forearms and fissuring of lips of child of twenty one months who had taken 200 000 to 300 000 I U of vitamin A daily Blood contained 330 I U per 100 ml of plasma

was checked when xanthosis cutis developed presumably due to its storage in the skin The level of vitamin A was raised to the upper limits of normal —126 to 153 I U per 100 ml —but not beyond

The level of carotene in the blood at which xanthosis cutis develops differs widely in different people Hoch [571] found in one experimental subject it was 375 micrograms and in another 470 micrograms per 100 ml In cases of established xanthosis cutis the levels have been bet
610 micrograms per 100 ml [570] the former f
limits [695]

The curious can
becom

The earliest and almost constant effect of excess of vitamin A is a dry rough itching skin with dry coarse sparse hair on the scalp eyebrows and eyelashes Growth and appetite remain normal though there may be some loss of weight There is no constipation or polyuria The child may be irritable and unhappy but this is a late symptom probably caused by the onset of pains in the limbs and feet which are the usual reason for the limping child being brought to a doctor

On examination the salient findings are very tender firm deep swellings in the forearms and less constantly in the legs feet and hands Such swellings are not hot or discoloured or attached to the skin Caffey [579] from very careful radiological examinations of seven children—some of whose pictures

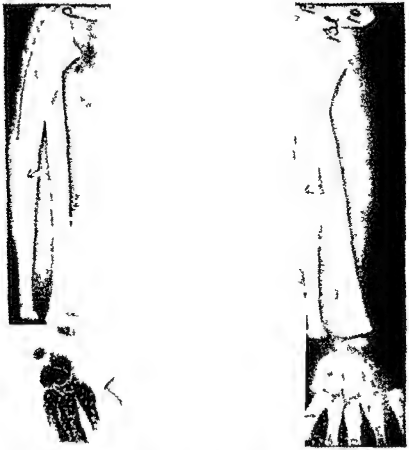


FIG 31 Hyperostoses of the ulna of the patient shown in Fig 30

he has most kindly allowed us to reproduce (Figs 27 32)—has shown that these swellings are cortical hyperostoses which have a shell like appearance with a zone of diminished density between the subperiosteal thin layer of bone and the external surface of the old cortex After withdrawal of vitamin A this intermediate clear zone disappears the hyperostoses then shrinks on to the old cortex and fuses with it These solid sclerotic cortical thickenings are in turn gradually resorbed from within but remain visible for many months after complete clinical recovery The constancy with which the ulnæ and metatarsals are involved—possibly due to their being exposed to trauma—*is an important diagnostic point*

Other but inconstant findings are an enlarged liver a raised sedimentation rate an increase in serum alkaline phosphatase and lipoids a decrease in serum protein and a moderate leucocytosis and anæmia The temperature and urine are normal though the latter has been reported to contain small

amounts of vitamin A The level of vitamin A in the blood has varied in different infants from 400 I U to 3,743 I U per 100 ml

Recovery when the vitamin is no longer given is surprisingly rapid within a week the child is no longer terrified of being touched and can run without pain The level of vitamin A in the blood, however, may remain raised for months and the liver, rarely, remains enlarged

The differential diagnosis from infantile cortical hyperostosis should not be difficult since this condition always appears before the fourth month of life and involves the face and jaw, while vitamin A poisoning has never been reported during the first year nor has it ever affected the face and jaw Further points of difference are that in infantile cortical hyperostosis the temperature is usually raised, the metatarsals are but rarely involved and the level of vitamin A in the blood is normal Other conditions such as

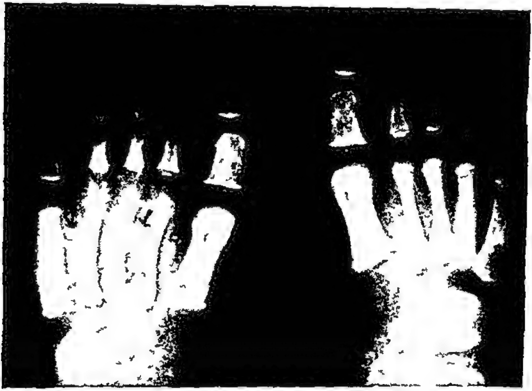


FIG 32 Hyperostoses of the metatarsals of the patient shown in Fig 30

scurvy (p 70), poisoning with vitamin D (p 578) tuberculosis syphilis and rheumatic fever are too unlike vitamin A poisoning to cause confusion if the possibility of the latter is borne in mind

In adults and older children only one doubtful case [603] of chronic vitamin A poisoning has so far been reported, though Spiesman [341] states that only 40,000 I U daily may cause general malaise with loss of weight and appetite But one of us often gives 144 000 I U daily for many weeks with no ill effects apart from an occasional and transient diuresis at the beginning of treatment, and doses of 300 000 I U daily for several months appear to be harmless for older children [467]

Hypervitaminosis A in Animals Rodahl [585] in 1950 published a most comprehensive monograph on hypervitaminosis A which includes not only a complete bibliography and summary of the work of others but also a detailed account of his own work on rats guinea pigs, mice rabbits and cockerels More recently Wolbach and his collaborators [587] also studied the condition in dogs In passing it is of interest that polar bears [577] are often found with septic wounds, various bony deformities and abnormalities and also

partially united fractures with large amounts of callus all of which could be caused by chronic vitamin A poisoning from the bear's habit of eating a whole seal at a meal, which would provide from 30 to 100 million I U of vitamin A

The symptoms of acute poisoning in rats are general malaise a staring coat, drowsiness muscular weakness and reduced activity even with a single dose as high as 1 500 000 I U death does not occur and no changes are found in the bones or internal organs

The symptoms of chronic poisoning come on after several days on doses of 200 to 500 I U per gram of body weight There is impaired appetite weakness loss of hair soreness of the skin swelling of the eyelids and exophthalmos limping and spontaneous fractures Biopsies show in most animals enlarged adrenals, degeneration of the renal tubules and general hyperæmia with extensive macroscopic and microscopic hæmorrhages throughout all the organs This hæmorrhagic tendency is in part due to the hypoprothrombinæmia which is caused by hypervitaminosis A and can be prevented by giving vitamin K [415] On the other hand hæmorrhages may occur with a normal prothrombin time and in spite of vitamin K [585]

The effect of vitamin A on ossification has already been discussed on p 43 so that here it is only necessary to add that Fell and Mellanby [586 609] have shown by *in vitro* experiments on the fœtal bones of mice that hypervitaminosis A acts directly on the bone itself and not by any indirect effect Maddock and Wolbach [588] claim that in rickets large amounts of vitamin A cause rapid repair of the metaphysis and resumption of the calcification of the cartilage matrix and osteoid tissue but Rodahl [585] found that far from vitamin A aiding calcification in rickets it decreases it and is also more injurious than it is to normal animals Though scurvy in guinea pigs produces lesions very similar to those caused by hypervitaminosis A yet only slight protection against excess of vitamin A is given by ascorbic acid and lack of ascorbic acid only slightly increases the toxicity of vitamin A

Fertility in hypervitaminotic rats is reduced though there are no pathological changes in the genital organs Normal young may be produced which have very high hepatic stores of vitamin A these young if suckled by their own mothers develop hypervitaminosis A and die

VITAMIN A_2

Vitamin A_2 is chiefly found in fresh water fish and is probably of little more than theoretical importance since it appears to be made from the same vegetable precursors as vitamin A_1 and to have the same biological functions Biological tests show that the pure vitamin [589] has a vitamin A activity of 1 300 000 U S P units per gram [590] or about forty per cent of the activity of crystalline vitamin A_1

Its structure according to Morton and others [591] is the same as that of vitamin A_1 except that it has an extra double bond situated in its ring Shantz [589] however after reviewing the evidence is dubious of this and all the other formulæ which have been suggested

The absorption spectrum of vitamin A_2 gives two bands with maxima at 350 and 288 millimicrons and with antimony trichloride a band at 693 with subsidiary bands at 660 and 635 640 millimicrons [149] the latter according to Lederer and Rothman [592] causing difficulty in the estimation of vitamin A_1 by overlapping its band at 620 millimicrons Cyclized vitamin A_2 gives bands at 391 369 349 334 millimicrons which are almost identical with those of cyclized vitamin A_1 [149 593] but the latter is not so well absorbed by alumina [593] Cyclization does not alter the antimony trichloride spectrum of vitamin A_2 [593] Shantz [589] using a purer preparation of vitamin A_2 gives very slightly different values to some of the above

Vitamin A_2 is found in all fish liver oils but those of fresh water fish contain most [149] In the latter there appears to be a fixed ratio between

vitamin A_1 and vitamin A_2, which is dependent on whether the fish is carnivorous, omnivorous, or migratory, but is not affected by age, sex, weight, or season [594]. Mammals, including man, birds and reptiles [595] do not store vitamin A_2 in their livers unless their diets, like that of the seal and otter which live on fresh water fish, are very rich in the vitamin [599, 600]. Rats and frogs have been found to store vitamin A_2 when it has been given in large quantities [594] and Milas [282] has reported finding it in the olfactory mucosa of the steer. The precursors of vitamin A_2 are probably the same as those of vitamin A_1, since Morton and Creed [596] showed that perch after being fed on leaf carotene formed both vitamins. Vitamin A_2 is probably not converted in the body to vitamin A_1 [590].

The functions of vitamin A_2 are apparently the same as those of vitamin A_1, since vitamin A_2 is biologically active when fed to rats [590, 599, 600], though it is more toxic [601] and less efficiently stored [590]. Wald [597] found that it entirely replaced vitamin A_1 in the visual purple cycle of fresh water fish. In migratory fish both vitamins are present in the eye, the proportion of vitamin A_1 being greatest in those fish which spawn in salt water [598]. Fluorescence microscopy is able to differentiate between the two vitamins in the tissues and examination under an ultraviolet lamp is an excellent way of separating livers which contain one or the other vitamin, vitamin A_2 giving a brownish orange instead of a brilliant yellow fluorescence [58].

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CHAPTER II

THE VITAMIN B COMPLEX

UNTIL 1926 it was generally believed that "vitamin B" was a single entity. In that year Smith and Hendrick [1] showed that it consisted of two factors, a thermolabile anti-neuritic factor and a thermostable growth promoting factor. After the dual nature of vitamin B had been demonstrated the American Society of Biological Chemists decided to call the factors *vitamin B* and *vitamin G* respectively. In England the names vitamins B_1 and B_2 were suggested. Vitamin B_1, or aneurine, was first isolated in 1926; its identity and synthesis took another ten years. It soon became evident that vitamin B_2, the thermostable factor, was a complex.

Considerable confusion has resulted over nomenclature. Thus the name vitamin B_2 was subsequently given to the vitamin now known as riboflavine, which is also called lactoflavine on the Continent, and was formerly known as vitamin G in America. The situation has been further complicated by the fact that workers in different laboratories have discovered factors independently and each group has given the factor its own name. For example, pyridoxine has been successively known as factor Y, factor I, factor H, adermin and vitamin B_6.

When the B vitamins were isolated as chemical compounds many were given chemical names indicating their nature, e.g. pantothenic acid, riboflavine, pyridoxine, etc., and these are used in the literature instead of the older names. The term "vitamin B complex" now refers to all the vitamins split off from the original "vitamin B" and identified chemically or by

FIG. 33. A case of vitamin B deficiency in a London woman before treatment. The photograph shows angular stomatitis and a red fissured tongue.

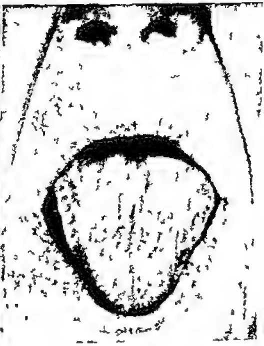


FIG. 34. Same case as in Fig. 33 after treatment with the vitamin B complex.

Fig. 35 A case of vitamin B deficiency in a London woman before treatment Presenting complaints depression, insomnia memory impairment red cracked lips glossitis dermatitis on exposed parts flexures and vulva, photophobia Poor dietary history

Fig. 36 Same case as Fig. 35 after several weeks treatment with the vitamin B complex

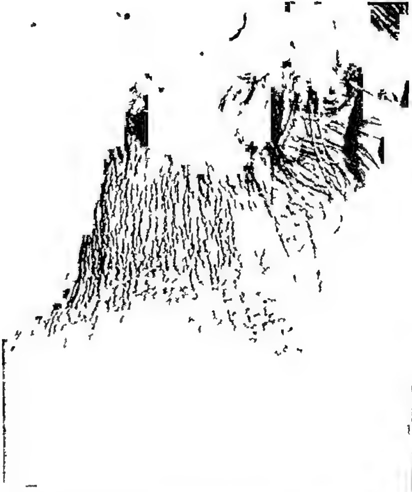


Fig 37 A case of vitamin B deficiency in a London woman before treatment Same case as Fig 35 showing scaly pellagroid dermatitis at back of neck

Fig 38 Same case as Figs 35 to 37 After treatment with the vitamin B complex

their biological effects A B vitamin has been defined as an organic substance which acts catalytically in all living cells and which is essential for the nutrition of higher animals [2]

The components of the vitamin B complex are

- Vitamin B_1, Aneurine or Thiamine (see p 183)
- Riboflavine, formerly Vitamin B_2 (see p 285)
- Nicotinic Acid (see p 333)
- Vitamin B_6
- Pantothenic Acid
- Biotin
- Folic Acid
- Vitamin B_{12}
- Folinic Acid or the Citrovorum Factor

In addition there are several substances of somewhat doubtful status as B vitamins They are

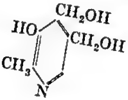
- Inositol
- Choline
- Para Aminobenzoic Acid
- "Vitamin B_{13}"
- "Vitamin B_{14}"

VITAMIN B_6

Isolation and Chemistry of Vitamin B_6 In 1934 György reported the existence of a factor distinct from the water soluble factors known at that time, lack of which caused dermatitis or acrodynia in rats It was called

FIG 39 Crystals of Vitamin B_6 (Pyridoxine).

vitamin B_6 and was later shown to be identical with the factor Y of Chick and Copping [63] the antidermatitis factor of Hogan and Richardson [64], the 'vitamin H' of Booher [65] and the factor I of Lepkovsky Jukes and Krause [66] The vitamin was isolated in 1939 by a number of investigators [69–71] and its structure determined in the same year [72 73] It is 2 methyl 3 hydroxy 4 5 dihydroxymethyl pyridine,



The compound was synthesized in 1939 by Harris and Folkers [76] in America and by Kuhn [77] and his co-workers in Heidelberg.

Since two other compounds related to pyridoxine, pyridoxal and pyridoxamine, show vitamin activity it is suggested that the term vitamin B_6 be used to signify the group.

Kuhn suggested the name adermin in 1938, and in the following year György and Eckhardt [79] proposed that it should be called pyridoxine, name which was adopted in 1940 by the Council on Pharmacy and Chemistr of the American Medical Association [80].

Pyridoxine forms colourless crystals, M.P. 206–208° C. (with decomposition), soluble in water and alcohol, stable to heat and alkali, but not to light, especially ultra-violet. It is more susceptible to the action of light in neutral and alkaline media [186]. The pH of a one per cent. solution of the hydrochloride is 2·44.

Chemical methods are generally inapplicable for assaying pyridoxine because they estimate pyridoxal and pyridoxamine which also have vitamin activity (p. 105). All three give different colours when treated with diazotized sulphanilic acid [3, 139]. Melnick [4] has modified the method for estimating pyridoxine only, by using borate. Microbiological methods depending on the stimulation of the growth of yeast and bacteria (*Streptococcus fæcalis* and *Lactobacillus casei*) are also used. The yeast *S. carlsbergensis* estimates total pyridoxal, pyridoxamine and pyridoxine; *S. fæcalis* estimates pyridoxamine and pyridoxal; and *L. casei* estimates pyridoxine only. It is thus possible by using these organisms to estimate all three of the B_6 vitamins [5–8]. A biological method involving the growth response of rats has been employed, but this estimates not only pyridoxine, but also pyridoxamine and pyridoxal [9, 10].

Distribution of Vitamin B_6 in Foods. Vitamin B_6 appears to be wide' distributed in foods. It is mainly present as pyridoxal and pyridoxamin in hydrolysed foodstuffs; very little pyridoxine is present. In plant materi pyridoxine occurs commonly with pyridoxal and pyridoxamine. Yeast liver, cereal polishings, cereals and pulses are particularly good sources. There is an increase of this vitamin in cereals on germination. Fish is a moderately good source; vegetables and milk contain little.

Vitamin B_6 Content ... *stuffs* [5]

Apples	
Bananas	
Bean, dried	
Beef, muscle	per gm.

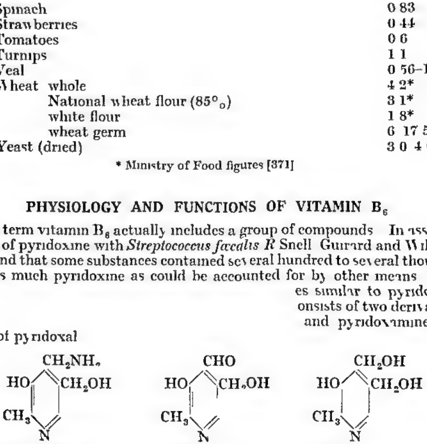
Vitamin B_6 Content of Foodstuffs [5]—continued

	M ograms per gm
Corn meal white (maize)	0 54
Eggs	0 22
Grape fruit	0 09
Halibut	1 1
Lamb leg	0 81
Mackerel	2 1 2 7
Marmite	4 0
Milk new	0 06
dried	0 50
Molasses	2 7
Mushrooms	0 45
Mutton shoulder	0 18
Onions	0 63
Orange	0 8
Oyster	0 33
Peas fresh	0 79 1 9
dried	3
Peanuts roasted	3
Pork loin	0 86 2 7
bacon	0 23 1 0
ham	0 19–1 7
Potatoes	2 2 3 2
Raisins	0 34
Royal jelly	2 4
Salmon	4 5
Sardine	1 6–2 8
Spinach	0 83
Strawberries	0 44
Tomatoes	0 6
Turnips	1 1
Veal	0 56–1 3
Wheat whole	4 2*
National wheat flour (85%)	3 1*
white flour	1 8*
wheat germ	6 17 5
Yeast (dried)	3 0 4 0

* Ministry of Food figures [371]

PHYSIOLOGY AND FUNCTIONS OF VITAMIN B_6

The term vitamin B_6 actually includes a group of compounds In assaying sources of pyridoxine with *Streptococcus faecalis R* Snell Guirard and Williams [17] found that some substances contained several hundred to several thousand times as much pyridoxine as could be accounted for by other means This
es similar to pyridoxine
]
onsists of two derivatives
and pyridoxamine the
amine of pyridoxal

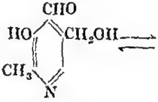


Pyridoxamine Pyridoxal Pyridoxine

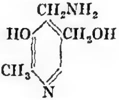
Harris, Heyl and Folkers [20] determined the structure and synthesis of these compounds. The work of Gunsalus and his co-workers [22] has clearly demonstrated that pyridoxal phosphate is the coenzyme for *amino-acid decarboxylase* (which removes the carboxyl group from amino acids).

$$X.CH(NH_2).COOH \longrightarrow X.CH_2.NH_2 + CO_2$$

and for *transaminase*, an enzyme catalysing transamination. The latter is the biological process of transferring amino groups from appropriate amino-acids to keto acids or compounds with carbonyl groups. The following scheme has been suggested but recent work by Gunsalus and his co-workers [22] does not confirm this.

$HO.CO.CH_2.CH_2.CH(NH_2).COOH$ +  $\rightleftharpoons$

Glutamic acid — Pyridoxal

$HO.CO.CH_2CH_2.CO.COOH$ + 

α-Ketoglutaric acid — Pyridoxamine

Only pyridoxal phosphate, and neither pyridoxamine phosphate nor pyridoxine phosphate, is active as a coenzyme [23]. Although the final proof of structure is lacking the active form of pyridoxal phosphate appears to be the alcoholic or 5-phosphate [23].

Reactions Catalysed by Pyridoxal Phosphate. 1. Amino Acid Decarboxylation. This is probably an important means by which bacteria can metabolize some amino acids. Pyridoxal phosphate has been conclusively shown to catalyse the following decarboxylations:

$R.CH(NH_2).COOH \longrightarrow R.CH_2.NH_2+CO_2$
Tyrosine $\longrightarrow$ Tyramine $+CO_2$
"Dopa" $\longrightarrow$ 3 : 4-Dihydroxyphenylethylamine
(Dihydroxyphenylalanine) (precursor of adrenaline) $+CO_2$
Lysine $\longrightarrow$ Cadaverine $+CO_2$
Ornithine $\longrightarrow$ Putrescine $+CO_2$
Arginine $\longrightarrow$ Argamine $+CO_2$
Glutamic acid $\longrightarrow$ 3-Aminobutyric acid $+CO_2$

This has been shown only in bacterial enzyme systems. It is not known whether pyridoxal phosphate functions in the decarboxylation of other amino acids in the mammal.

2. Transamination. Pyridoxal phosphate is a coenzyme for the glutamic acid—aspartic acid and the glutamic acid—alanine systems:

(i) $\underbrace{HO.OC.CH_2.CH_2.CH(NH_2)COOH}_{\text{Glutamic acid}} + \underbrace{HO.OC.CH_2CO.CO.OH}_{\text{Oxalacetic acid}}$

$\rightleftharpoons \underbrace{HO.OC.CH_2.CH_2.CO.CO.OH}_{\text{1-Ketoglutaric acid}} + \underbrace{HO.OC.CH_2.CH(NH_2).CO.OH}_{\text{Aspartic acid}}$

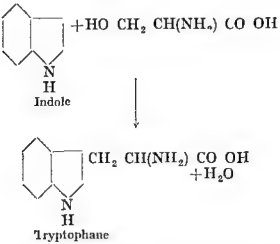
(ii) $\underbrace{HO.OC.CH_2.CH_2.CH(NH_2).CO.OH}_{\text{Glutamic acid}} + \underbrace{CH_3.CO.CO.OH}_{\text{Pyruvic acid}}$

$\rightleftharpoons \underbrace{HO.OC.CH_2.CH_2.CO.CO.OH}_{\text{1-Ketoglutaric acid}} + \underbrace{CH_3.CH(NH_2).CO.OH}_{\text{Alanine}}$

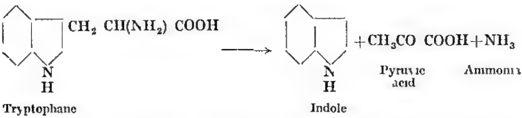
Another transaminase catalysing a glutamic cysteic acid system is known

The rate of transamination in the tissues of animals deficient in vitamin B_6 is considerably diminished [694]

3 **Synthesis and Degradation of Tryptophane** Pyridoxal phosphate catalyses the synthesis of tryptophane from indole and serine [27]



The formation of indole from tryptophane by *B coli* proceeds as follows [27]



Vitamin B_6 and Amino Acid and Protein Metabolism The metabolism of tryptophane in animals and man is known to be altered profoundly in pyridoxine deficiency large amounts being excreted as xanthurenic acid [29 45] the amount of the latter excreted being proportional to the quantity of tryptophane in the diet [106] Reid and his co workers [41] studied the excretion of xanthurenic acid in pyridoxine deficient rats to determine the pathway of tryptophane to xanthurenic acid Of the various compounds fed to rats only L tryptophane and kynurenine appeared to yield xanthurenic acid the administration of which was diminished by administering vitamin B_6 The metabolic pathway is apparently tryptophane$\longrightarrow$kynurenine$\longrightarrow$ xanthurenic acid Pyridoxal may play a part in the metabolism of tryptophane possibly by hydrolytic removal of the side chain The possibility exists that the further metabolism of xanthurenic acid requires vitamin B_6 and that in the presence of the latter kynurenine is metabolized without the formation of xanthurenic acid as an intermediate to nicotinic acid [49] or to anthranilic acid and alanine [107] Tryptophane or casein which contains considerable quantities if administered in large amounts to the rat or mouse accentuates a deficiency of vitamin B_6 [28] High protein diets also accentuate a vitamin B_6 deficiency probably because of the increased intake of methionine and other thioamino acids rather than of tryptophane [106] Vitamin B_6 can prevent the depression of growth due to excessive doses of methionine [705]

Rats suffering from a prolonged vitamin B_6 deficiency are unable to convert tryptophane to nicotinic acid [42] There is no direct evidence that

vitamin B_6 plays a role in the conversion of tryptophane to nicotinic acid in man, it is involved however, in its conversion into indole acetic acid like compounds [46] The vitamin B_6 deficient monkey shows deranged tryptophane metabolism and increased excretion of xanthurenic acid [154]

The observations of Sadhu and Brody [332] suggest that the specific dynamic action of an amino acid depends not only on its nature but also on the presence of vitamin B_6 which functions as a prosthetic group in the catalytic system for transamination and on the presence of α ketonic acids such as pyruvic acid which function as amino acceptors

There is an increased excretion of urea ammonia, uric acid and creatinine in vitamin B_6 deficient animals and a lowered blood urea [56] A lowered blood urea has also been observed in patients suffering from hyperemesis gravidarum and this has been returned to normal levels by giving vitamin B_6 [78] It has therefore been suggested that a vitamin B_6 deficiency or insufficiency occurs in the vomiting of pregnancy although it is equally possible that metabolic changes occur associated with a low blood urea

Recent work has linked vitamin B_6 to reactions involving the ' unnatural D amino acids Some lactic acid bacteria show increased ability to utilize D in place of L amino acids when grown in a medium rich in vitamin B_6 [81] Armstrong and her co workers [82] have shown that in the vitamin B_6 deficient rat the administration of D amino acids causes the excretion of a large amount of dietary nitrogen that would otherwise be utilized

The sparing action of alanine on the vitamin B_6 requirements of some organisms was formerly presumed to be due to its utilization in the synthesis of pyridoxal [86] It is now known that alanine is not a precursor of vitamin B_6 but that it is a product of its catalytic activity or can spare some metabolite produced by the activity of vitamin B_6 [90]

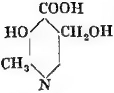
Cerecedo and his associates [126] conclude that in vitamin B_6 deficiency there is an abnormal metabolism of cystine and methionine Pyridoxal phosphate is a co factor in enzyme systems essential for the biosynthesis of cysteine [703] There may be a connection between the integrity of the epidermis and the normal metabolism of cystine and methionine This could explain the dermatitis occurring in vitamin B_6 deficient animals

The ability to convert protein to carbohydrate is diminished in dogs on a diet deficient in vitamin B_6 This is shown by a decreased dextrose nitrogen *ratio in phloridzinized animals on a high protein diet* the ratio returns to normal when the animals return to a diet adequate in vitamin B_6 [95] An increase in the protein intake in the diet of the rat increases the requirement of vitamin B_6 [19]

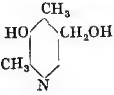
Fat Metabolism Some of the early observations on vitamin B_6 deficiency related this vitamin to fat metabolism particularly of the unsaturated fatty acids [86 96] There is no biochemical explanation of this in terms of the
reactions catalysed by pyridoxal pl
in fat or vitamin B_6 they develop
is relieved both by ethyl linoleate
or by vitamin B_6 [87 118] Very large d
also relieve the condition [121] Gavin
administration of vitamin B_6 aneurine
to an increase in body fat The same w
essential for the synthesis of fat from protein [163]

Adrenal Cortical Damage and Water Metabolism Pyridoxine deficiency produced by diets containing no pyridoxine or by feeding pyridoxine antagonists produces adrenal cortical damage which is *reflected in a disturbance* of water metabolism [693]

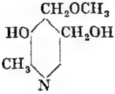
Analogues and Antagonists A metabolite of pyridoxine pyridoxic acid or 2 methyl 3 hydroxy 4 carboxy 5 hydroxymethylpyridine occurs in human urine [85] It is the chief metabolic product of either pyridoxine pyridoxal or pyridoxamine



Pyridoxic acid



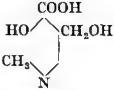
Desoxypyridoxine



Methoxy pyridoxine

As with other vitamins several analogues of vitamin B_6 are known that are antagonistic to its action Desoxypyridoxine or 2 4 dimethyl 3 hydroxy 5 methylol pyridine [104] administered to the chick rat mouse monkey dog and man produces symptoms of vitamin B_6 deficiency It also interferes with reproduction in the rat [123] Almost one hundred per cent mortality occurs in chick embryos treated with 1 mg of the compound this effect is prevented by simultaneous injection of vitamin B_6 into the egg [105] At least eight other analogues inhibit vitamin B_6 activity The most active of these is methoxypyridoxine or 2 methyl 3 hydroxy 4 methoxymethyl 5 hydroxymethylpyridine [107] which produces deficiency symptoms in the chick dog and monkey Approximately four molecules of this analogue counteract the response to one molecule of pyridoxine

Absorption Storage and Excretion On most natural diets most of the vitamin B_6 is ingested as pyridoxal or pyridoxamine [129] It is rapidly absorbed from the digestive tract of man dog and the rat Little is known about the destruction of vitamin B_6 in the tissues Small amounts of pyridoxine pyridoxal and pyridoxamine may be excreted in the urine but the major product of metabolism is 4 pyridoxic acid or 2 methyl 3 hydroxy 4 carboxy 5 hydroxymethylpyridine [341]



Pyridoxic acid

This is the chief metabolic product of pyridoxine pyridoxal or pyridoxamine [131] Much of the earlier studies on the excretion of vitamin B_6 were made before it was appreciated that it occurs naturally as a complex and that it may be excreted in any of the four forms just mentioned The method used for assay was not specific for pyridoxine and all the material estimated by this method was considered to be pyridoxine Although the recovery of ingested vitamin B_6 in the rat was fifty to seventy per cent [122] only ten to twenty per cent of the dose was recovered when pyridoxine was fed to dogs or to human subjects [120] Using microbiological assay methods by means of which pyridoxine pyridoxal and pyridoxamine could be estimated [129] Rabinowitz and Snell [131] studied the excretion products of three male subjects on a normal die ... ferent forms of vitamin B_6 Regardless of wh ... r pyridoxamine were given the chief excretion ... Pyridoxal gave rise to significantly larger quar ... did pyridoxine or pyridoxamine Neither pyridoxal nor pyridoxamine were converted to pyridoxine When pyridoxamine was administered both pyridoxal and pyridoxamine were excreted in approximately equal amounts and the administration of pyridoxine greatly increased the amount of pyridoxal and pyridoxamine excreted The excretion of all products was at a peak

at two to five hours after administration of the compound and returned to normal values after eight to twelve hours The highest recovery was seventy per cent when pyridoxal was given forty five per cent of the pyridoxine was recovered but only thirty one per cent of the pyridoxamine All forms of vitamin B_6 give rise to pyridoxal *which suggests that this is the form* used in metabolic processes (i e as pyridoxal phosphate) A major portion of each form is eventually oxidized to 4 pyridoxic acid

According to Linkswiler and Reynolds [681] vitamin B_6 is synthesized by man It is presumably synthesized by the tissues and not intestinal organisms *as excretion is greater than the intake even when sulpha drugs are administered to sterilize the gut* According to Lossy Goldsmith and Sarett [704] the excretion of vitamin B_6 in man does not seem to depend on the general level of nutrition

Requirements Vitamin B_6 is known to be an essential vitamin for many species *It is probably essential for man since deficiency symptoms have been* produced in human subjects by administering the antimetabolite desoxy pyridoxine (p 109) and these symptoms have been made to disappear rapidly by giving pyridoxine [84] Probable requirements calculated from those of different animal species may be of the order of 1 5 to 2 mg daily The *requirements are increased in experimental hyperthyroidism* [162]

Vitamin B_6 Deficiency Symptoms in Animals *Skin Lesions* Rats fed on diets deficient in vitamin B_6 develop rat acrodynia characterized by a symmetrical dermatitis affecting first the paws then the tips of the ears and nose which become red swollen and œdematous The matted fur on the backs of the hind paws desquamates leaving a denuded pale pink glistening skin Sometimes there is fissuring or ulceration at the corners of the mouth and over the tongue [26 62] Other species show deficiency symptoms but only the rat suffers from acrodynia It is considered by some workers that rat acrodynia is not a specific sign of vitamin B_6 deficiency as they have failed to repeat these observations others have prevented it by administering the essential fatty acids (p 674)

Nerve Lesions As far back as 1938 Chick and her co workers [89] ob served that rats kept on diets deficient in vitamin B_6 for periods of more than four months suffered from epileptiform convulsions which could be prevented by administering vitamin B_6 *Similar convulsions have also been* rep [illegible] [99] and p gs [188 194] These convulsions can be prevented by [illegible] king part in certain biological transamina tio[illegible] hich vitamin B_6 is a necessary enzyme It is also known that glutamic acid is essential for the metabolism of brain tissue so that *it is possible that the epileptiform convulsions* occurring in vitamin B_6 deficient animals may be due to a derangement of a glutamic acid metabolism

Wintrobe and his associates [193 195] and Swank and Adams [187] investigated the patholog cal changes in the nervous system of pigs suffering from vitamin B_6 *deficiency The animals* developed stiffness of the hind legs eventually resulting in ataxia with loss of tendon reflexes and failure to respond to painful stimuli motor activity was not impaired Degenera tive changes were observed in the peripheral nerves spinal roots posterior root ganglia sensory ganglia and in the posterior columns of the sp nal cord the brain stem and brain showed no changes At the time the possible relation of these lesions to those seen in the nervous system in pernicious anæmia was commented upon It is now known that the latter is in no way associated with a deficiency of vitamin B_6 *Davenport and Davenport* [141] have shown that vitamin B_6 deficiency decreases the electrical convulsion threshold which rises in deficient animals but not in normals after the administration of vitamin B_6 Glutamic acid also increases the threshold but tryptophane which intensifies vitamin B_6 deficiency lowers it In severe deficiency pyridoxine causes only a slow rise in the electrical convulsion

threshold unless glutamic acid has been administered previously These facts suggest that maintenance of transaminase activity is critical for a high electrical convulsion threshold

Hæmatological Changes Vitamin B_6 deficient animals become anæmic This has been demonstrated in the dog, pig and monkey [92, 93, 112, 140, 153, 164, 194, 736] A mild anæmia also develops in the deficient chick [145], accompa
small spl
chromic
grams pe
shifted to the left and an irregular reticulocytosis may appear The bone marrow is hyperplastic Hæmosiderosis of the spleen, liver, and bone marrow has been reported [194] Elevated plasma iron levels have been observed in dogs [93, 164] In the vitamin B_6 deficient pig, iron is absorbed but not utilized for hæmopoiesis [331] The anæmia in deficient animals responds to treatment with vitamin B_6 although some investigators maintain that this vitamin alone is insufficient [112] It does not respond to iron In human subjects kept on diets deficient in vitamin B_6 there was no evidence of anæmia [84]

Anæmia due to a deficiency of vitamin B_6 has some of the features of Mediterranean anæmia (thalassæmia, Cooley's anæmia) In both the red cells are hypochromic and microcytic, the serum iron is raised, and iron containing pigment is found in the tissues Vitamin B_6 is of no value in the treatment of human anæmia of nutritional origin, or the anæmia of nephritis, aplastic anæmia, pernicious anæmia or the anæmia of infection [150]

Various changes in the white cells have been recorded In the dog and monkey there is a leucocytosis, an absolute increase in the number of circulating neutrophils and an absolute decrease in the number of leucocytes [153 161]

Vitamin B_6 deficiency in the mouse results in agranulocytosis and lymphopenia [166], this also occurs in the mouse with transplanted leukæmia In the monkey vitamin B_6 deficiency produced by administering desoxypyridoxine causes atrophy of the thymus, lymph nodes and bone marrow, leucopenia and lymphopenia [419] The efficiency of phagocytosis by the leucocytes in the blood of animals deficient in vitamin B_6 is seriously impaired [345]

The anæmia of vitamin B_6 deficiency is not due to interference with tryptophane synthesis (p 107), because anæmia can be produced by a deficiency of this amino acid and it does not resemble that due to vitamin B_6 deficiency [150]

A high incidence of poikilocytosis in certain dairy cattle in Michigan has been observed [191] It is stated to disappear by feeding supplements of

In the rhesus monkey vitamin B_6 deficiency

sclerosis [154 156]
material in the intr
of collagen and elastic fibres in the mucinous matrix (Figs 40 and 41) The close resemblance between the pathology in the two conditions does not prove, however, that vitamin B_6 deficiency is necessarily concerned in the genesis of human arteriosclerosis

deg
[21
defi
hæmaturia, occur in pyridoxine deficient rats [695] Complement titre and the formation of specific immune bodies (γ globulin) are disturbed during the early stages of vitamin B_6 deficiency in rats [179]

Vitamin B_6 deficiency has been produced in rats by feeding excess of aneurine and flour of high extraction rate (seventy per cent.) [184, 185].

Human Vitamin B_6 Deficiency. Hawkins and Barsky [208] attempted to produce vitamin B_6 deficiency in human volunteers by feeding diets deficient in the vitamin, but the period of deprivation was only two months and was probably not long enough. The only changes observed were a rise in the total white cell count and a decrease in neutrophils. The mental confusion and depression observed could have been due to the monotonous diet. Gellhorn and Jones [188] made similar attempts to produce a deficiency by administering desoxypyridine to six patients with lymphosarcoma and acute leukæmia. Two patients had epileptiform convulsions, but there was no true evidence of vitamin B_6 deficiency as there was no abnormality of tryptophane metabolism as measured by the xanthurenic acid excretion [189].

Mueller and Vilter [190] succeeded in producing deficiency symptoms in eight subjects given doses of 60 to 150 mg. of desoxypyridoxine and a diet poor in the vitamin B complex. Seborrhœa-like skin lesions developed about the eyes, nose and mouth, and erosions appeared about the mouth resembling ariboflavinosis. Glossitis and stomatitis resembling the lesions of nicotinic acid deficiency (sore, swollen, red tongue and buccal mucous membrane) were also observed in some of the patients, one of whom developed nausea, vomiting, weakness and dizziness. These symptoms could well have been due to the toxic effect of the desoxypyridoxine. The lesions did not respond to riboflavine, nicotinamide or aneurine, but disappeared within three days of administering pyridoxine. The ratio of desoxypyridoxine to pyridoxine was approximately 1 : 1. The only change of note in the hæmatogram was a mild, absolute lymphopenia. Hypochromia, siderosis and the other changes seen in animals suffering from vitamin B_6 deficiency (p. 110) were not observed nor were xanthurenic acid or kynurenine found in the urine of any of the patients unless large quantities of tryptophane were administered [735].

Toxicology and Pharmacology. Vitamin B_6 is relatively non-toxic even in large amounts [111]. Rats tolerate doses up to 1 gram per kilogram of body weight; above this dose convulsions appear. The lethal dose for this animal is 4 to 6 gram per kilogram by mouth. The repeated administration of 100 mg. per kilogram of body weight is well tolerated. Toxic effects have never been reported in man.

The effect on the blood sugar in man is variable. Doses of 20 to 800 mg. intravenously may produce a rise or a fall in normal subjects [119]. Even minute amounts of vitamin B_6, e.g. 0·00001 to 1·0 mM/litre significantly increase the total work output of perfused muscle.

Antibody formation is defective in animals on diets deficient in vitamin B_6 [179, 197] and there is evidence that susceptibility to infection, e.g. pneumonia, is increased [205].

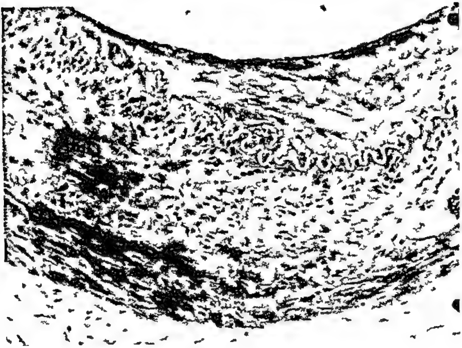
THERAPEUTIC USE OF VITAMIN B_6

Vitamin B_6 has been used clinically in a wide variety of conditions, but on the whole the results do not suggest that the vitamin has any therapeutic action. A deficiency syndrome has not been observed, except in human subjects purposely deprived of the vitamin.

Deficiency States. Spies and his colleagues [108, 109] state that 50 mg. of vitamin B_6 given parenterally produces considerable subjective improvement in patients suffering from deficiency diseases such as pellagra and beriberi. They observed relief of symptoms and increased muscular power.

Blood Diseases. In some respects vitamin B_6 deficiency anæmia resembles pernicious anæmia and thalassæmia (Mediterranean anæmia). Vilter and his co-workers [110] state that vitamin B_6 produces a reticulocytosis in nutritional macrocytic anæmia and in pernicious anæmia. Kark [94], however, failed to observe any improvement in six anæmic patients receiving vitamin

VITAMIN B_6 DEFICIENCY IN THE MONKEY



FIG

elastic membrane

FIG 41 Vitamin B_6 deficiency in the monkey Weigert Van Gieson stain × 80 Abdominal aorta showing newly formed elastic tissue in the thickened intima The changes in Figs 40 and 41 are arteriosclerotic and bear a close resemblance histologically to those seen in human arteriosclerosis

B_6 It is generally considered that vitamin B_6 is of no value in the treatment of pernicious anæmia or Mediterranean anæmia [150]

Following a report of the occurrence of a leucocytosis in cases of anæmia treated with vitamin B_6, Cantor and Scott [209] treated three patients with granulocytopenia (agranulocytosis) due to drugs with 200 mg of vitamin B_6 intravenously daily with apparently beneficial results Recovery was said to have occurred in forty-eight hours. Fishberg and Vorzimer [210] claimed that vitamin B_6 was a powerful leucopoietic stimulant in a case of granulocytopenia due to thiouracil These observations have not been confirmed Knutson and his colleagues [211] treated eight cases of granulocytopenia due to benzene and one due to amidopyrine, but could observe no beneficial action Menten [212] treated children suffering from granulocytopenia due to sulphonamides with folic acid and vitamin B_6 without effect Negative results were also obtained by Bandh [213] Death or recovery occurs in granulocytopenia and death is due to the sepsis which follows it The concomitant use of antibiotics, such as penicillin, has done much to reduce the mortality from the disease

Neuromuscular and Nervous Diseases. Spies and his associates [108, 109] noticed an increase in muscular strength in patients given vitamin B_6 parenterally. Accordingly Antopol and Schotland [114] administered it to patients with pseudohypertrophic muscular dystrophy in doses of 100 to 500 mg weekly and reported considerable improvement in muscular power Spies [109] claimed to have obtained improvement in patients with epilepsy, amyotrophic lateral [illegible]
vitamin Rosenbau[illegible]
weakness in patients [illegible]
hyperthyroidism, ulc[illegible]
treated 169 patients [illegible]
intrathecally in doses of 10 to 50 mg often with aneurine at the same time Among the diseases treated were poliomyelitis, general paralysis, tabes, disseminated sclerosis, Sydenham's chorea, neuritides of varying ætiology, and meningomyeloradiculitis Stone claims that the patients experienced subjective improvement, increase in muscle strength, improvement in gait and relief from pain when present Investigations by a number of other investigators have failed to confirm the results of these workers [243–250]

Spies [109] has used vitamin B_6 in eleven selected cases of Parkinsonism of at least four years' duration, eight of the cases being arteriosclerotic and three post-encephalitic. In the latter considerable improvement was reported within a few minutes; rigidity was significantly decreased and the patients walked without their usual stiffness Two of the arteriosclerotic patients showed definite improvement, five were unchanged and one was considerably worse From a study of forty six cases Jolliffe [115] concluded that no improvement occurred in postencephalitic cases and little or no improvement in patients hospitalized for over three years, but that a dramatic improvement resulted in approximately twenty per cent of cases of non-postencephalitic Parkinsonism that had been helpless for less than a year All patients received 50 to 100 mg of vitamin B_6 intravenously Jolliffe [200] subsequently extended the series to ninety cases and reported permanent improvement in nine, i e ten per cent. Baker [142] has also observed improvement in eight cases out of nineteen and Meller [171] in nine cases out of ten receiving a similar dosage They both state that in idiopathic paralysis agitans vitamin B_6 th[illegible] Three cases of paralysis agitans were also [illegible] 72] with doses of 50 to 100 [illegible] They noted subjective [illegible] strength Zeligs [143], and [illegible] [172] ten cases [illegible] vitamin [illegible] lso failed

to observe any benefit in twelve chronic cases The reported material is still too meagre for final appraisal but even accepting Jolliffe s latest report it would appear that vitamin B_6 therapy is only effective in ten per cent of patients with paralysis agitans a low figure that makes its use hardly worth while The introduction of such efficient drugs as the antihistamines artane and kemadrin has now made these observations of historical interest only

Vilter, Aring and Spies [116] describe a case of arsenical peripheral neuritis treated with vitamin B_6 in doses of 20 mg intravenously Improvement occurred and the patient relapsed when treatment was withdrawn

Schwartzman and his colleagues [170] treated three cases of Sydenham's chorea with 9 to 60 mg of vitamin B_6 daily and reported good results Improvement was rapid and progressive This was confirmed by Kost [97]

As vitamin B_6 deficient animals suffer from epileptiform convulsions, Fox and Tullidge [168] treated a number of epileptics with vitamin B_6 in doses of 20 to 100 mg daily for several weeks No improvement was noted

Skin Diseases Infantile seborrhœa has been treated with vitamin B_6 but the results obtained are not convincing [169] Jolliffe [203] treated a number of patients suffering from adolescent acne with oral doses of 50 to 250 mg of vitamin B_6 daily He reports that of thirty seven patients nine were cured and nineteen improved in a control series of thirty five, only seven improved Wright and his colleagues [204] described the treatment of an unspecified number of patients suffering from seborrhœic dermatitis and eczematous eruptions with 20 to 100 mg of vitamin B_6 daily The patients were stated to show a rapid response no details, however, are given

Vomiting Vitamin B_6 has been used in the treatment of nausea and vomiting in pregnancy and resulting from irradiation Many of the observations on the use of vitamin B_6 in the treatment of nausea and vomiting of pregnancy have been uncontrolled [206 207 342] Treatment consisted of injections of 50 to 100 mg daily or smaller doses more frequently Hesseltine [174] controlled his observations with injections of normal saline which he stated gave better results than vitamin B_6 Dorsey [178] claims that excellent results are obtained by the combined use of vitamin B_6 and adrenal cortical extract No controls were used nor were the results compared with any other form of treatment The influence exerted by psychic factors sedation and bed rest in the treatment of the vomiting of pregnancy cannot be overemphasized Owing to the lack of controls and the tendency for the condition to improve spontaneously such reports should be treated with considerable reserve

Bergmann [196] claimed similar beneficial results in the prevention of post anæsthetic nausea and vomiting with 100 mg vitamin B_6 given post operatively Only twelve cases were presented Hill [199] and Kernis and Stodsky [202] failed to confirm this on a study of 120 patients

Many therapeutic measures have been tried in the treatment of radiation sickness The presence of malnutrition and avitaminosis seems to increase lack of tolerance to radiation therapy There have been several reports on the use of vitamin B_6 in the prevention and treatment of radiation sickness [215 218 220 224 230 239] Some of these have been well documented and controlled and the total number of patients treated is well over 500 Wells and Popp [239] obtained the best results by giving 100 to 200 mg intravenously before each treatment, larger doses being given when treatment was directed to the abdomen, thorax or pelvis In most instances it was found that symptoms disappeared or were definitely relieved in ninety per cent of the cases Control tests were done to eliminate the possibility of psychic effects Nabarro [270] however, failed to observe any beneficial effect from treating patients subjected to irradiation with vitamin B_6

Patients suffering from neoplastic diseases such as Hodgkin's disease and leukæmia treated with nitrogen mustards and other mitotic poisons

invariably experience nausea and vomiting According to some workers this can be prevented by administering vitamin B_6 in doses of 100 mg parenterally one and a half hours after the administration of the nitrogen mustard [250 251]

Goldfeder and his co workers [237] investigated the effect of vitamin B_6 on radiation injury, resulting from X ray exposure in mice It had no appreciable effect on the hæmopoietic system but given before and after irradiation it significantly prolonged the survival rate of the animals as compared with irradiated but untreated controls

Other Conditions Vitamin B_6 is stated to have an anti histamine like action In man the skin reaction to histamine is slightly reduced by previous administration of the vitamin [217]

PANTOTHENIC ACID

History, Isolation and Chemistry The discovery of pantothenic acid had its origin in 1901 in the discovery of *bios* a hypothetical substance now known to be a mixture necessary to the reproduction of yeast In 1930 a pellagra like dermatitis occurring in chicks on restricted diets was described [47] and it was subsequently shown that this can be prevented by feeding pork liver and by a factor in liver extract [48] which was subsequently termed the "filtrate factor" Nutritional achromotrichia (greying of the fur) of rats fed diets deficient in one or more of the B vitamins has been observed by several investigators Morgan and his co workers [22] were the first to observe that the active substance which prevents and cures this greying of hair in rats on diets deficient in the B vitamins is present in the filtrate factor A collateral line of research was begun by Williams [256] who found that a naturally occurring compound of unknown composition stimulates the growth of yeast and to this compound he gave the name "pantothenic acid" on account of its ubiquitous distribution (Greek *παντος* everywhere) In 1939 Williams [50] isolated the compound and with Major [51] determined its structure in the following year It was found to be 1 hydroxy 2 2 dimethyl butyryl β alanine

$$\begin{array}{l} \qquad\qquad\quad CH_3 \\ \qquad\qquad\quad\; | \\ HO\ CH_2{-}C{-}CH(OH)\ CO\ NH\ CH_2\ CH_2\ COOH \\ \qquad\qquad\quad\; | \\ \qquad\qquad\quad CH_3 \end{array}$$

The identity of the chick anti dermatitis factor and pantothenic acid which had been suggested because of their similarity in chemical behaviour [53] was confirmed biologically by Jukes and others [55] In 1940 pantothenic acid was synthesized by several groups of investigators in America Switzerland and Germany [257 259] Macrae and his co workers [340] showed that the filtrate factor contains pantothenic acid and subsequently the latter was shown to be identical with the anti grey hair factor of former investigators [91 133]

Pantothenic acid shows both acidic and basic properties It is readily soluble in water and acetic acid slightly soluble in ether but almost insoluble in the other fat solvents (benzene chloroform) In a pure condition it is a yellow viscous oil It is unstable being sensitive to heat and to changes in the pH of the medium and is easily hydrolysed Pantothenic acid forms a well defined stable calcium salt which is the form in which it is marketed

Distribution Pantothenic acid is widely distributed in foodstuffs Yeast liver kidney wheat bran and peas are the best common sources royal jelly contains about six times as much as yeast [260] Pantothenic acid is synthesized by certain moulds bacteria and green plants and it also appears to be synthesized in the gut of ruminants Grains are good sources of panto

thenic acid but in the case of wheat about fifty per cent is removed in the milling process A considerable increase in the pantothenic acid content of cereals occurs on germination About eighty per cent of the pantothenic acid in foodstuffs is in the bound condition from which it can be liberated by *enzymic digestion with diastase and papain or by hydrolysis with acid* or alkali

The distribution of pantothenic acid in various foodstuffs has been determined by Waisman Mickelsen and Elvehjem [59] using a biological technique The stewing of meat reduces the pantothenic acid potency to one third probably through loss in the cooking water In the cooking dehydration and curing of meat about three quarters of the pantothenic acid is retained [355] Snell [60 290] and his co workers have elabor ated a biological method of assay depending on the growth response of *Lactobacillus casei* Methods depending on the growth rate of *Neurospora* [67] and *Lactobacillus arabinosus* [288 155] have also been devised [67] Schmidt [222] and Neilsen Hartelius and Johansen [272] employ *Streptobacterium plantarum*

Pantothenic Acid Content of Foodstuffs

	Micrograms per gm
Apples	0 60
Apricots (canned)	0 95
Artichoke	4 0
Asparagus	1 65
Bananas	0 7 1 8
Barley	10
Beans dried	8 3
baked (canned)	0 55 0 85
Beef muscle	4 9 15
liver	40 76
heart	20
brain	18
Beets	1 1
Bread whole wheat	5 7
white	4 6
Broccoli	11 14
Cabbage	1 8
Carrot	2 0 2 5
Cauliflower	9 2
Cheese	1 3 9 6
Chicken	5 3 6 2
Chocolate	1 9
Corn	3 1
Eggs	8–48 Av 27
Egg yolk	50 100 Av 63
Grapefruit	2 9
(canned)	1 2
Halibut	1 5
Kale	3 0
Lamb leg	6 0
Lettuce	1 1
Mackerel	3 1 4 7
Maize	3 1
Marmite	62
Milk	1 3 4 7 Av 2
skim	1–4 3
Molasses	2 6
Mushrooms	17

Pantothenic Acid Content of Foodstuffs—continued

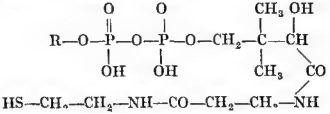
	Micrograms per gm
Mutton, shoulder	43
Oats	11
Onions	1 3
Oranges	0 7–3 4
Oyster	4 9
Peaches (canned)	3 5–4 5
Pears	3 5–4 5
Peas, fresh	3 8–10 4
dried	21
Peanuts, roasted	25
Pineapple (canned)	0 85
Pork, muscle	4 7–11
bacon	2 8–9 8
ham	3 4–6 6
Potatoes	3 2–6 5
Prunes	0 6
Pumpkin	4 0
Raisins	0 9
Rice	4
Rice bran	15–27
"Royal jelly"	89 ; 511
Salmon	6 6–10
Sardines (canned)	4 7–6 0
Shrimp	2 5
Soya bean	18
Spinach	1 2–1 8
Strawberries	2 6
Tomatoes	1 0–3 7
(canned)	2 0–3 7
Tuna fish	4 2
Turnips	0 37
Veal	1 1–2 6
Walnut	8 0
Wheat, whole	5 1–11 0
germ	7 0–8 5
bran	2 4
Flour, white (70% extraction)	3 0
(85% extraction)	4 48
Yeast	140–350 Av. 200

These figures are probably much too low, owing to incomplete hydrolysis before assay of the pantothenic acid. Neilands and Strong [54] have elaborated a procedure for complete hydrolysis of bound pantothenic acid in foodstuffs

Pantothenic acid is present in foods in a bound form Two such forms, coenzyme A [98] and pantothenic acid conjugate [99], have been extensively purified [100] The form in which pantothenic acid is bound is unknown, but it is probably with proteins or amino-acids through the amide linkage

Biogenesis. Although a relatively small number of bacteria synthesize pantothenic acid, bacterial synthesis is an important source of the vitamin in nature It is produced, for example, in the gut of the ruminant [101] Milk contains more pantothenic acid than can be accounted for in the dietary intake Synthesis also occurs in the cæcum of the rat, and may account for as much as sixty per cent. of ... Some organisms, such as *C. diphtheriæ* ... ic acid

Functions and Physiology **Coenzyme A** Pantothenic acid is the prosthetic group of a coenzyme concerned in acetylation known as coenzyme A In 1942 an enzyme was discovered in brain tissue necessary for the synthesis of acetylcholine from acetic acid choline and adenosine triphosphate [103] A few years later it was shown that an enzyme in liver acetylates sulphonamides and other amides [135] and a coenzyme was isolated and shown to be a necessary component of the system which acetylates choline Coenzyme A is a derivative of pantothenic acid and adenosine and has been tentatively assigned the following formula [159 346]



R is an adenosine residue According to King and Strong [483] coenzyme A is a phosphate not a pyrophosphate Coenzyme A probably brings about acetylations by accepting an acetyl group at its terminal—SH the resulting thioacetyl coenzyme transferring its energy rich acetyl group to substate molecules Plasma pantothenic acid is in the form of the free vitamin whereas that in the red blood cells is in the form of coenzyme A Although all the reactions catalysed by coenzyme A involve a common substrate they result in the formation of a variety of compounds including amides esters anhydrides and compounds produced by the condensation of an acetate radical with keto acids or acid phosphates

Reactions in which Coenzyme A is Co factor *in vitro*

Formation of Acid Phosphates
Acetic acid+ATP—Phosphoryl acetyl intermediate+ADP

Formation of Esters
Choline+acetic acid+ATP—Acetylcholine+ADP+phosphoric acid

Formation of Amides
p Aminobenzoic acid (or sulphonamides)+acetic acid+ATP=
acetylated PABA or sulphonamide+ADP+phosphoric acid

Formation of Hydroxy Acids from Keto Acids
Oxalacetic acid+acetic acid+ATP=Citric acid+ADP+phosphoric acid

Condensation of Acetic Acid
2 Acetic acid+ATP—Acetoacetic acid+ADP+phosphoric acid

Reactions Occurring *in vivo* **Requiring Pantothenic Acid**

Acetic acid⟶Fatty acids (bacteria)
Acetic acid⟶Sterols (bacteria)
Acetic acid⟶Aromatic amino acids (bacteria)

$$\left.\begin{array}{l}\text{Acetic acid}\\ \text{Glucose}\\ \text{Pyruvic acid}\\ \text{Lactic acid}\end{array}\right\}\longrightarrow CO_2+H_2O \qquad \text{(bacteria)}$$

Proteins or carbohydrates⟶Fats (rat)

The catalytic function of coenzyme A in citric acid synthesis has been confirmed in experiments with cell free extracts of yeast and bacteria [234] The reaction in which oxalacetic acid is converted to citric acid is the one which initiates the cycles by which both carbohydrates and fatty acids are metabolized aerobically When carbohydrate metabolism furnishes the acetyl group aneurine and pantothenic acid are required When fatty acids or alcohol are the source pantothenic acid only is needed to initiate the reaction The synthesis of fats from carbohydrate or protein involves

processes in wl . . .
coenzyme A
of the organis
oxidation of those amino acids that are converted to pyruvic acid [255] Protein has a pantothenic acid sparing action in the rat [254]

Pantothenic Acid Deficiency. Pantothenic acid is essential for the adequate nutrition of a number of species, including rats, dogs, mice, chicks, hogs and monkeys It is not known whether it is essential for human nutrition as no definite deficiency symptoms have ever been described in man

Rats maintained on a diet deficient in pantothenic acid develop a deficiency syndrome characterized by scant, coarse fur, inflammation of the nose, staining of the fur with porphyrin, failure to grow, hæmorrhage and necrosis of the adrenals and gastric ulceration [58, 294] Anæmia and leucopenia also occur [125, 275], and inflammatory changes in the lungs have been described [278] Corneal vascularization has been observed [292] Dogs on deficient diets show a fatty degeneration of the liver, a rise of blood sugar, ... enteritis, and diminished carbo... on [183, 231] Pantothenic acid ... ogs, in which deficiency of the vitamin results in an ulcerated gastro intestinal tract, emaciation, incoordination (goose stepping gait), loss of hair, rhinorrhœa, a scaly dermatitis, and degenerative changes in the peripheral nerves, posterior roots, and the posterior columns of the spinal cord [271, 296] The animals also suffer from a normocytic anæmia In the monkey the pantothenic acid deficiency syndrome shows lack of growth, ataxia, greying and thinning of the fur, anæmia, diarrhœa and cachexia [279] Chicks suffering from pantothenic acid deficiency d ... the spinal cord [123, 269] I ... the growth of the skeleton, ... ndochondral ossification [295]

Pantothenic acid deficiency in the experimental animal reduces the phagocytic power of the blood [345] and antibody response [268] but the animals are less susceptible to certain infections such as pneumococcal pneumonia and poliomyelitis [151, 227], presumably because the organisms concerned require pantothenic acid as a growth factor

Nutrition of Micro organisms Pantothenic acid is essential for the nutrition of a large number of micro organisms, both pathogenic and non pathogenic, and some organisms have been used for the microbiological assay of the vitamin (p 117) It is essential for the growth of *Streptococcus hæmolyticus*, *Diplococcus pneumoniæ*, *Proteus morgagnii*, *Clostridium tetani*, *C Welchii* certain strains of *Corynebacterium diphtheriæ*, some species of *P* ... rial parasite *P* ...
... c acid from β ... amin in the intestine The excretion of pantothenic acid in the urine and fæces exceeds the intake in the diet, indicating that bacterial synthesis occurs in the gut [282] In ruminants this pantothenic acid synthesized by bacteria is probably utilized, but it is not certain whether it is in man

Pantothenic Acid Antagonists Many compounds are known that interfere with the synthesis of pantothenic acid by micro organisms or its utilization by animals Sulphapyridine
tion in rats by producing
salicylates will also prevent
acid [160], probably by interf

$$CH_2OH.C(CH_3)_2\ CHOH\ COOH$$

which when coupled with β alanine forms pantothenic acid

... of pantothenic acid have been prepared that can ... blocking its participation in essential ... these to be prepared was pantoyltaurine, ...

pantothenic analogues were prepared when it was discovered that the malarial parasite *P lophuræ* requires pantothenic acid as a growth factor [274] Panthenol, the alcohol of pantothenic acid, is almost as active as pantothenic acid Phenylpantothenone, $CH_2OH\ C(CH_3)_2CHOH\ CO\ NH\ CH_2CH_2COC_6H_5$ is a pantothenic acid antagonist, preventing its utilization by bacteria [236] ω Methyl pantothenic acid produces a pantothenic acid deficiency in mice [691] Many other antagonists have been prepared, the most active being those in which the pantoic acid part of the molecule is coupled to a suitable amino acid amino ketone amino alcohol or amine

Absorption, Storage, Excretion of Pantothenic Acid Pantothenic acid is absorbed from the gastro intestinal tract In human blood it is stated to be present in quantities carrying from 3 7 to 40 micrograms per 100 ml [44, 158, 181] Following the intravenous injection of sodium or calcium pantothenate the blood concentration rises by about fifty per cent in three hours in normal subjects, but not in patients suffering from deficiency diseases [61] Most of the pantothenic acid is present in blood in the combined form as it is precipitated with protein precipitants When pantothenic acid is injected into human subjects there is a rise of blood riboflavine as well, and conversely administration of riboflavine causes a rise in blood pantothenic acid Pantothenic acid is partly destroyed in the body, that escaping destruction being excreted in the urine or stored

The pantothenic acid content of different human tissues has been estimated by Nielsen and others [272] who give the following figures in micrograms per gram of dry material muscle, 4, liver, 40, kidney, 30, spleen, 20, nerve, 3, pancreas, 7, adrenals, 5, and stomach, 10

Pantothenic acid is excreted in the sweat, milk and urine The amount excreted in the sweat is stated to vary ... [262 263] Human milk contains 48 m... increasing to 245 micrograms by the f... 200 to 250 micrograms per 100 ... with intakes of 6 0 to 9 5 ... nine per cent is excreted ... to human milk

The average daily excretion of pantothenic acid in man varies from 1 5 ... ine [176] Sarett [147] ... and Denko *et al* [223] ... mg is excreted in the fæces After ingestion of 100 mg the excretion rises to 18 5 mg, and to 38 5 mg after the intravenous administration of this amount Schmidt [222] gives the daily urinary excretion of pantothenic acid as 2 72 ± 0 61 mg in men 2 63 ± 0 6 mg in women and 2 05 to 2 86 mg in children He attributes the higher values of other authorities to failure to sterilize the urine, which ... Injection ... henic acid, ... in normal ... n child the excretion ranges from 99 to 218 micrograms [228] Schmidt [222] Gershberg and others [241] investigated the excretion of pantothenic acid by patients suffering from ... chylia ... s) but ... which

processes in which reactive acetyl molecules condense under the influence of coenzyme A Pantothenic acid also functions in the pyruvic acid metabolism of the organism *Proteus morgagni* [265] Pantothenic acid stimulates the oxidation of those amino acids that are converted to pyruvic acid [255] Protein has a pantothenic acid sparing action in the rat [254]

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Many organisms e g *E coli*, can synthesize pantothenic acid from β alanine, and this would appear to be the source of the vitamin in the intestine The excretion of pantothenic acid in the urine and fæces exceeds the intake in the diet, indicating that bacterial synthesis occurs in the gut [282] In ruminants this pantothenic acid synthesized by bacteria is probably utilized, but it is not certain whether it is in man

Pantothenic Acid Antagonists Many compounds are known that interfere with the synthesis of pantothenic acid by micro organisms or its utilization by animals Sulphapyridine will produce achromotrichia or lack of pigmentation in rats by producing an induced pantothenic acid deficiency [280], salicylates will also prevent the growth of bacteria utilizing pantothenic acid [160], probably by interfering with the synthesis of pantoic acid,

$$CH_2OH\ C(CH_3)_2\ CHOH\ COOH$$

which when coupled with β alanine forms pantothenic acid

A number of analogues of pantothenic acid have been prepared that can act as antagonists to the vitamin, blocking its participation in essential enzyme actions One of the first of these to be prepared was pantoyltaurine, $CH_2OH\ C(CH_3)_2\ CHOH\ CO\ NH\ CH_2CH_2SO_2OH$, which inhibits the utilization of pantothenic acid by organisms such as *S hæmolyticus*, *C diphtheriæ* and *D pneumoniæ* [271] and also by animals [273] A large number of pantothenic analogues were prepared when it was discovered that the malarial parasite *P lophuræ* requires pantothenic acid as a growth factor [274] Panthenol, the alcohol of pantothenic acid is almost as active as pantothenic acid Phenylpantothenone, $CH_2OH\ C(CH_3)_2CHOH\ CO\ NH\ CH_2CH_2COC_6H_5$ is a pantothenic acid antagonist, preventing its utilization by bacteria [236] ω Methyl pantothenic acid produces a pantothenic acid deficiency in mice [691] Many other antagonists have been prepared the most active being those in which the pantoic acid part of the molecule is coupled to a suitable amino acid amino ketone amino alcohol or amine

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The average daily excretion of pantothenic acid in man varies from 1 5 to 7 mg or 70 to 600 micrograms per 100 ml of urine [176] Sarett [147] states that the normal excretion averages 3 5 mg daily and Denko *et al* [223] give the figure 2 68 to 3 46 mg , about 0 89 to 3 66 mg is excreted in the fæces After ingestion of 100 mg the excretion rises to 18 5 mg , and to 38 5 mg after the intravenous administration of this amount Schmidt [222] gives the daily urinary excretion of pantothenic acid as 2 72 $\pm$ 0 61 mg in men, 2 63 $\pm$ 0 6 mg in women and 2 05 to 2 86 mg in children He attributes the higher values of other authorities to failure to sterilize the urine, which acts as a growth medium for bacteria synthesizing pantothenic acid Injection of 25 mg of pantothenol, the alcohol corresponding to pantothenic acid, increases the excretion of pantothenic acid to from 4 5 mg to 6 7 mg in normal

, In the newborn child the

Schmidt [222] Gershberg

ntothenic acid by patients

l cœliac disease, achylia,

aplastic anæmia hepatitis, pneumonia, Addison's disease, diabetes) but found that it was within normal limits, except in pernicious anæmia, in which

there was diminished excretion. The excretion of pantothenic acid is not increased after the injection of β-alanine, suggest[illegible] is not capable of synthesizing pantothenic acid [[illegible] sulphathalidine, have no effect on the excretion [illegible] adults [357]

Pharmacology. The toxicology of pantothenic acid was investigated by Unna and Greslin [136]. The LD_{50} following subcutaneous injection is 2·7 grams per kilogram in mice and 3·4 grams in rats. The daily administra[illegible]

[illegible] logical changes in the organs. Blood pressure, respiration and heart rate were uninfluenced by doses of 10 to 50 mg. per kilogram and a ten per cent solution was not irritant to the rabbit conjunctiva. Spies and his co-workers [61] administered 100 mg. intravenously to human subjects without producing any side effects. They observed that the administration of *riboflavine* causes an increased excretion of pantothenic acid. It is stated that the intravenous injection of 100 mg. calcium pantothenate causes a rise of blood sugar in normal and diabetic subjects [157].

The total work output of the frog gastrocnemius muscle is significantly increased by perfusion with 0·01 mille mols. of calcium pantothenate per litre [367]. The effect is probably due to vasomotor changes and it does not follow that a similar result would be obtained in the intact animal or in man.

Requirements of Pantothenic Acid. The daily requirement of pantothenic acid needed to prevent achromotrichia in rats is 50 to 100 micrograms [91, 133]. The requirements of other species have been calculated but those of man are entirely speculative. It has been assumed that it is an essential constituent of human diet but absolute proof is lacking. It has been estimated that an average American diet contains 4·5 mg. of pantothenic acid per 2,500 calories. Human excretion figures suggest that the daily requirement for man might be about 10 mg. This figure, however, is entirely speculative, those figures obtained from dietary studies being more reliable. A good mixed diet *must* contain all the vitamins and in required amounts for man.

Clinical Studies on Pantothenic Acid. The production of nutritional achromotrichia in animals by dietary means and the restoration of normal colour by feeding pantothenic acid led to the hope that it might be effective in the treatment of human grey hair. There are two fallacies here; one is that similar symptoms do not necessarily have a common cause, the other is that the findings from animal experiments cannot be transferred to human conditions. It has never been shown that dietary deficiency is a factor in the production of grey hair in the human adult. Widespread publicity was given in the popular press to the possibility of pantothenic acid curing human greyness. Subsequent investigation proved that no authenticated case of human greyness has responded to treatment with pantothenic acid [276, 277, 366]. Schmidt [692] observed no difference in the excretion of pantothenic acid in patients with grey hair and baldness and normal individuals. Nicholls [198] and Hughes [358] believe, however, that greying of hair can occur in native children in the tropics owing to malnutrition. Hughes found achromotrichia widespread among malnourished children in Lagos usually associated with hypopigmentation, and he believes that it is due to pantothenic acid deficiency, since the administration of the vitamin seemed to restore the normal colour. As a good diet was administered at the same time it is difficult to ascribe the greyness solely to a deficiency of pantothenic acid.

The "burning feet" syndrome, although previously described in the literature on deficiency diseases, has recently received considerable attention on account of its frequent occurrence in Japanese prison camps in the last

war (see p. 320) According to Gopalan [359] the daily injection of 20 to 40 mg of calcium pantothenate produced spectacular improvement in patients suffering from this syndrome. A similar syndrome was observed by Paraita [360] in the Spanish Civil War of 1936–39 and although pantothenic acid was not discovered then Paraita accepts the conclusions of Gopalan that this syndrome is due to a deficiency of the vitamin B_2 complex particularly pantothenic acid. This view is also accepted by Smitskamp [361]

Cases of glossitis and cheilosis are stated to have healed following the administration of pantothenic acid after treatment with other members of the vitamin B complex had proved ineffective [364, 365] Doses of 150 to 300 mg daily were used

Panthenol, the alcohol of pantothenic acid, has been suggested as an adjunct to the pituitary adrenocorticotrophic hormone and cortisone in the treatment of acute disseminated lupus erythematosus and alone in the treatment of subacute lupus erythematosus and chronic discoid lupus erythematosus [368, 738] Welsh [732] claims that vitamin E and pantothenic acid, or its alcohol, are also effective

BIOTIN

History, Isolation and Chemistry. The discovery of biotin, also known as vitamin H and coenzyme R, had its origin in the casual observation that a high concentration of egg white in experimental diets is toxic, some years

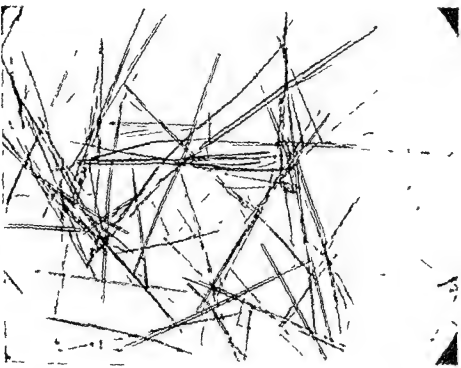
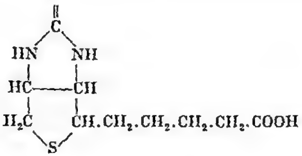


Fig. 42 Crystals of biotin Magnification × 150

later certain foods, notably liver and yeast, were discovered which contained an organic substance capable of protecting rats against the toxic effects of egg white, or "egg white injury" The protective factor was called "the protective factor against egg white injury" and also "vitamin H" by György in 1931 It was extensively studied by György and his co workers [10–16] between 1931 and 1940 In another laboratory attempts were being made to resolve "bios," a yeast growth factor, and this resulted in the isola-

tion in 1936 of a crystalline substance from egg yolk by Kögl and Tonnis [281], who named it biotin. About a milligram of active material was obtained from a quarter of a ton of dried egg yolk. Independently in yet another laboratory attempts were being made to isolate a compound, essential for the growth of *Rhizobium*, a nitrogen-fixing organism; this was called "coenzyme R" [474]. It was not realized by these various laboratory groups that they were dealing with the same entity. In 1940 Gyorgy and his associates [16] announced the identity of biotin with vitamin H and coenzyme R. Biotin was subsequently isolated from liver [146], its structure established by Du Vigneaud and his co-workers [283] in 1942, and its synthesis achieved in 1943 by Harris and his colleagues [284] in the Merck laboratories in the United States.

Biotin is water- and alcohol-soluble, but relatively insoluble in the fat solvents. It crystallizes in long thin needles (Fig. 42), melting at 232–233° C. with decomposition. It is heat stable, and not decomposed by acids or alkalis, although it is inactivated by hydrogen peroxide and rancid oils and fats. Biotin is a bicyclic urea derivative containing sulphur in a thiophene ring. It is D-2′ keto-3 : 4-imidazolido-2-tetrahydrothiophene-*n*-valeric acid,



A series of papers has been published by Kögl and his co-workers which state that the biotin isolated from egg yolk is not the same as that isolated from liver [362]. It has a m.p. of 220° C. The two compounds, which differ in physical and chemical properties, have been designated α-biotin and β-biotin, respectively. The formula above is that of β-biotin obtained from liver.

No chemical or physical methods are known for the estimation of biotin. The bioassay, based on the cure of egg white injury in rats and chicks is time consuming, and has been replaced by microbiological methods depending on the growth response of yeast [287], *Lactobacillus helveticus* [290], *L. arabinosus* [288] and other organisms [285].

Units and Distribution. Until the isolation and identification of biotin, biological units were employed to denote the potency of biotin-containing material. The "rat unit" of biotin is the daily dose of a standard preparation which will cure egg white injury produced in rats on a special diet; this corresponds to 0·1 micrograms of biotin or 10,000 units per milligram of biotin [293]. The "yeast growth" unit [281] is the amount of biotin producing a one hundred per cent. increase in cell growth of a specified strain of yeast under certain conditions; this corresponds to 27,000 yeast growth units per milligram [293].

Biotin is found in foodstuffs containing other members of the vitamin B complex, particularly yeast, liver, kidney, and other offal, light chicken meat, eggs, peas, cocoa and cereals. An increase in the biotin content of cereals occurs on germination. Biotin is present in most foodstuffs in a bound form, from which it is liberated in the intestine by enzymic hydrolysis [291]. An average of seventy-seven per cent. of the biotin is retained in meat after cooking [329]. The biotin content of some common foods is given in the following table :—

Biotin Content of Foodstuffs

	Micrograms per g
Apples	0.003
Bananas	0.044
Beans, dried	0.098
Beef muscle	0.026
Beets	0.027
Bread, whole wheat	0.019
white	0.011
Cabbage	0.024
Carrot	0.025
Cauliflower	0.17
Cheese	0.036
Chicken	0.054–0.098
Chocolate	0.32
Corn	0.058
Eggs	0.030
Grape fruit	0.030
Halibut	0.08
Lamb leg	0.021
Lettuce	0.031
Mackerel	0.03–0.18
Maize	0.058
Marmite	0.001
Milk, cow	0.011–0.037
human	0.038–0.08
Molasses	0.091
Mushrooms	0.16
Mutton shoulder	0.027
Onions	0.035
Oranges	0.019
Oyster	0.087
Peas, fresh	0.035
dried	0.18
Peanuts, roasted	0.34
Pork muscle	0.02–0.046
bacon	0.075
ham	0.04–0.06
Potatoes	0.006
Raisins	0.031
Royal jelly	1.7–4.1
Salmon	0.15
Sardine	0.04–0.24
Spinach	0.069
Strawberries	0.040
Tomatoes	0.040
Tuna fish	0.03
Turnips	0.021
Veal	1
Wheat, whole	0.081
flour, white	0.02–0.047
Yeast (Torula utilis)	1.33
(Brewers')	0.071

Avidin and Biotin Inhibitors. The syndrome produced in rats by feeding large quantities of raw egg white first described in 1927 by Boas [286] is in effect the syndrome of biotin deficiency. There exists in egg white a basic protein that combines with biotin and renders it unavailable to the body forming a complex with it that is not utilized [307]. egg white also inhibits

the *utilization of biotin by micro organisms* [308] The isolation and properties of this protein in egg white which has been called avidin has been described and its analysis effected [149 309] It is a glycoprotein and the complex it forms with biotin cannot be broken down by proteolytic digestion only by heating or irradiation This explains why raw egg white inactivates biotin whereas *cooked egg white does not* The combination between biotin and avidin is stochiometric and the biotin cannot be separated by dialysis It is peculiar however that although the avidin biotin complex cannot be broken down by digestion the biotin is utilized if the complex is injected [311] Biotin deficiency can be induced in animals and in man (p 129) by feeding raw egg white

A number of analogues of biotin have been prepared that act as physiological antagonists to biotin preventing its utilization by animals and micro organisms Biotin deficiency can in fact be produced by administering such compounds They act not by combining with biotin like avidin but by blocking essential enzyme mechanisms in which biotin plays a part *Desthio* biotin in which the sulphur atom of biotin is replaced by hydrogen is a biotin inhibitor [306] Biotin sulphone [475] although a growth factor for yeast is a biotin inhibitor for *Lactobacillus helveticus L arabinosus* and *Staph aureus* Some other analogues are known with biotin activity although less than that of the parent substance e g oxybiotin or O heterobiotin in which the sulphur atom is replaced by oxygen [177]

Physiology and Functions of Biotin Biotin is essential for the growth of many bacteria yeasts and fungi although some organisms are capable of synthesizing it [298] Bacterial synthesis occurs in the gut of animals [299] and also in the human gut as the combined excretion in urine and fæces is greater than the intake [301] The vitamin appears to be related to the fundamental process of growth since the biotin content of embryo tissues *and tumour tissue is very high* [148]

Biotin has been shown to activate the reversible deamination of aspartic acid and the deamination of serine and
the ability of *B coli* to produce carbon
and oxaloacetic acid it has been conc ...
coenzyme of oxaloacetic decarboxylase [313] By analogy with other vitamins it was suspected *that it does not function in these reactions as such* but is converted into a coenzyme form Lichstein [314] has shown that biotin probably exists in a coenzyme form *in natural materials It has been* shown that the failure of certain micro organisms to grow on a biotin deficient medium is due to their inability to condense carbon dioxide with pyruvic acid to form oxalacetic and aspartic acids Using radio active carbon (C_{14}) as a tracer element Lardy and his co workers [315] have shown that biotin may be associated with the fixation of carbon dioxide in several different enzyme reactions in animals as well as micro organisms Thus in presence of biotin *L arabinosus* fixed C_{14} into cellular aspartic acid rats were better able to fix C_{14} into adenine guanine arginine aspartic acid citric acid *and bone* carbonate if they were supplied with adequate biotin

Biotin may be concerned with the oxidation of pyruvic acid and lactic acid since carbon dioxide production is markedly increased in systems in which tissues are respiring in solutions containing pyruvate acid lactate as *substrate* [369] *This has been confirmed* using radio active carbon (C_{14}) as a tracer element [369]

It has been suggested that biotin plays a part in fat metabolism as fatty livers are produced in animals by feeding biotin [*305*] *There may also be* a connection between biotin deficiency and pantothenic acid since symptoms of biotin deficiency induced in rats by administering succinylsulphathiazole are aggravated in the presence of a deficiency of pantothenic acid [318] Biotin appears to be necessary for the maintenance of normal creatine levels in the muscle of the rat [320]

Metabolism, Absorption and Excretion Biotin is absorbed from the gastro intestinal tract and freed from the bound form in which it occurs in foodstuffs The amount excreted daily by human subjects on a normal diet varies from 11 to 183 micrograms a day [301 322 324] It is not influenced by disease and even during periods of starvation is not abnormally low although it does depend on protein intake [285] Sydenstricker [322] noted that the excretion fell to 3 5 to 7 3 micrograms daily on a biotin deficient diet The average diet may supply from 4 to 170 micrograms of biotin daily the amount is increased if liver is included The excretion may increase from 245 to 357 micrograms after a tolerance dose of 500 micrograms of biotin by mouth [267] Biotin is also excreted in the fæces to the

Fig e days received since the age of desquamative The dermatitis

extent of about 322 to 393 micrograms daily and as this is greater than the intake intestinal synthesis and absorption must occur [68] It has been shown that biotin can be absorbed from the colon [267] This intestinal synthesis is inhibited by large doses of non absorbable sulphonamides such as sulphasuxidine although other sulpha drugs in normal doses do not seriously interfere [267] Several organisms are probably responsible for the synthesis the following are known to produce it *B coli B proteus vulgaris B fæcalis alcaligenes B mesentericus* Only small amounts are excreted by human subjects on diets containing large amounts of egg white [301] The exact nature of the excretion product is not known, some of the biotin present does not combine with avidin [301] The excretion of biotin is said to be low in patients with seborrhœa [267 330] The amount excreted in milk is low, particularly just after parturition In mature milk

FIG 44 Section of Skin from Case of Suspected Human Biotin Deficiency. It shows loose sheets of keratinized epithelium, absence of rete pegs and cellular infiltration around the hair follicles, sweat glands and blood vessels. The corium is thickened, hair follicles atrophic and sebaceous glands absent. See text, p 130.

FIG 45 Section of Skin from Case of Suspected Human Biotin Deficiency It shows a marked cellular reaction around the small blood vessels in the corium See text, p 130

the concentration has been estimated to be two to fourteen per cent of the intake and varies from 0 9 to 11 2 micrograms in twenty four hours [328]

Pharmacology [335 337] Biotin is non toxic even in large amounts A single intravenous injection of 1 gram per kilogram produces no toxic effects in mice It produces no significant signs of irritation applied to the cornea or injected intramuscularly or intradermally In the anesthetized animal it has no effect even in large doses on blood pressure respiration hepatic function renal function metabolic rate or intestinal circulation It has no effect on the regeneration or healing of tissue

Effects of Biotin Deficiency in Animals Biotin deficiency can be produced in animals by feeding diets containing much raw egg white or by administering sulphonamides

In rats biotin deficiency is characterized by a generalized erythematous scaly greasy pruritic dermatitis arrest of growth an abnormal posture and spastic gait (Fig 43) and a typical atrophy of fur around the eyes producing the condition known as spectacle eye Degenerative changes have also been described in the thymus testes and epididymis skin and muscles [316] and alopecia may occur [370] When biotin deficiency is induced by feeding sulphaguanidine or succinylsulphathiazole granulocytopenia leucopenia and anemia develop as well [303 304] Hyalinization and necrosis of voluntary muscle and sclerosis and calcification of the blood vessels also occur The stress of lactation may produce a mild biotin deficiency in the rat the rat requires biotin for gestation and lactation [338]

In the mouse biotin deficiency causes all the symptoms described in the rat and alopecia and greyness in mice with dark fur [343] It is essential for reproduction and lactation in mice [344]

Cattle do not need an exogenous supply of biotin which is synthesized by bacteria in the rumen [319]

Biotin deficiency in the chick results in dermatitis and perosis [317] the vitamin is necessary for normal embryonic development of the egg [299] The pig with biotin deficiency suffers from alopecia spasticity cracks in the skin of the extremities and a dry rough dermatitis [300] In the monkey biotin deficiency is characterized by thinning of the fur and pigmentary changes in the hair [347] The biotin deficient puppy suffers from a progressive paralysis [349] This is probably due to a secondary potassium deficiency since it is reversed by potassium

Biotin and Infection It has been claimed that biotin activates lysozyme the lytic enzyme in tears mucus sputum and body fluids that digests bacteria [374] This however has been disputed [375] A deficiency of biotin increases the severity of infection with *Plasmodium lophuræ* in chicks and infection of rats with *Trypanosoma lewisi* [377 380] Biotin deficient rats are more susceptible to infection with *Salmonella typhimurium* [356]

Biotin Deficiency in Man Sydenstricker and his colleagues [175] have reported a deficiency syndrome in four volunteers kept on a diet poor in biotin lack of which was accentuated by including large quantities of egg white in the diet Vitamin supplements were added so that the diet was adequate in all other respects At the beginning of the experiment all the volunteers were in good condition and free from symptoms and signs of avitaminosis During the third and fourth weeks all developed a fine scaly dermatitis which disappeared spontaneously After the seventh week one volunteer developed a maculosquamous dermatitis of the hands arms and legs During the seventh and eighth week all showed a striking greyish pallor of the skin which was interpreted as a sign of vasoconstriction Eventually all the volunteers developed a definite atrophy of the lingual papillæ which was either general or patchy with the production of a geographical tongue The tongue remained pale and in no way resembled that seen in ariboflavinosis (p 315) or pellagra (p 358) By the ninth and tenth week all showed dryness of the skin of the extremities with a tendency to fine branny desquamation

After the fifth week many of the symptoms associated with aneurine deficiency (p 239) began to appear—mild depression to extreme lassitude muscle pains, hyperesthesia, localized paræsthesiæ, anorexia and nausea Blood examinations showed a fall in hæmoglobin percentage, the number of erythrocytes and volume of packed red cells, these changes occurred in spite of an adequate iron intake There was a striking rise of bile pigments and cholesterol in the blood The urinary excretion of biotin fell to 3 5 to 7 3 micrograms in twenty four hours Treatment with an injectable biotin concentrate, 75 to 300 micrograms daily, resulted in relief of signs and symptoms in three to five days The minimal amount of biotin for prompt relief was 150 micrograms daily The urinary excretion rose from 3 to 7 micrograms to an average of 55 micrograms of biotin daily

A case of suspected biotin deficiency has been reported by Williams [321] The patient was an old retired Italian labourer, who suffered for years from an exfoliative dermatitis and mild conjunctivitis There was nothing in the history of the case to account for the rash, but the dietary history was significant Since adolescence the patient had a passion for raw eggs, the consumption of which ran into six dozen weekly The whites of twelve eggs contain sufficient avidin to bind 230 micrograms of biotin [322] Therefore, it would seem that little if any biotin would be absorbed from the gastro intestinal tract His choice of foods was narrow, and excluded good sources of biotin, the diet included one to four quarts of wine daily The skin lesion, which was studied by biopsy, did not correspond to that due to a deficiency of nicotinic acid, riboflavine, pantothenic acid or pyridoxine but closely resembled that seen in animals with biotin deficiency Half the total surface of the body appeared normal, but the entire face, ears, shoulders, dorsum of forearms, hands and lower legs were a fiery red and covered with scales Skin biopsy showed loose sheets of keratinized epithelium, flattened rete pegs, and cellular infiltration around the hair follicles, sweat glands and blood vessels (Figs 44 and 45) The epidermis showed hyperkeratinization and parakeratinization, and the corium was thickened, due to interstitial œdema and hypertrophy of the collagen fibres Sebaceous glands were absent, hair follicles atrophic, and sweat glands and ducts dilated The macroscopic and microscopic changes of the skin were compatible with a diagnosis of biotin deficiency, although not pathognomonic of the condition as seen in animals Before treatment the serum biotin was low After the patient had been hospitalized for a fortnight on a liberal diet and given injections of biotin methyl ester, the dermatitis largely disappeared, the serum biotin returned to normal levels, and the patient's general condition improved

Brown [221] described three cases of infants who had a mild skin lesion after the administration of sulphonamides for respiratory infections This lesion resembled those described by Sydenstricker in volunteers fed on raw egg white (p 129) The lesions were aggravated by administering egg white and improved by biotin methyl ester According to Berger [330] the excretion of biotin in infants with seborrhœa is lower than normal, the effect on the seborrhœa of administering biotin is not mentioned Oppel [267] also recorded a low response in patients with seborrhœa to a tolerance test with biotin, but he was unable to observe any beneficial effects from administering biotin

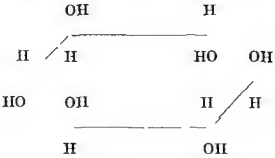
Nutritional deficiency in children is sometimes associated with changes in the texture and colour of the hair (p 368) Chavarria and his co workers [illegible] young children [illegible] was thought to accelerate the growth and return [illegible] olour, although the authors are careful to point out that the observations were uncontrolled and not fully proven

Biotin and Cancer. If butter yellow (*p* dimethylaminoazobenzene) is

fed to rats hepatic tumours are produced although their formation is delayed by diets rich in protein or some members of the vitamin B complex This protective effect is prevented by administering biotin [334] Tumour tissue, including carcinomata also contains abnormally high concentrations of biotin [289] It was therefore considered that biotin might be essential for the growth of neoplastic tissue and that by diminishing the biotin intake of cancer patients the growth of existing tumour tissue might be inhibited Kensler and his co workers [37] did not note any beneficial effect in mice with mammary carcinoma fed a diet rich in egg white and Kline and his colleagues [35] failed to observe a decrease in liver tumours in affected rats kept on diets deficient in biotin The inoculation of fragments of sarcoma 37 or sarcoma 180 into mice already suffering from biotin deficiency is followed by tumour growth occurring at the expected rate [39] Rhoads and Abels [323] gave large quantities of egg white to two cancer patients in the hope of arresting the growth of the neoplasm There was no improvement in their clinical condition and there was no evidence of biotin deficiency although the egg white contained sixteen to forty times enough avidin to bind the biotin of the diet Kaplan [333] treated a group of ten cancer patients maintained on diets low in biotin with the whites of thirty six to forty two eggs daily but no beneficial effect on the disease was noted although it was considered that the general condition of the patients was markedly improved

INOSITOL

In 1940 Woolley [138] showed that inositol was essential for the growth of hair in the mouse deficiency leads to alopecia Since then it has been shown to be required for the nutrition of several other species Although inositol has been known to be a cyclohexanehexanol since 1887 its configuration has only more recently been established as meso cyclohexanehexanol with the following configuration



Meso inositol

Inositol is widely distributed in large amounts in most animal and plant tissues fruits and cereals being good sources Yeast and crude liver extracts contain a considerable amount In animal tissues it appears to be combined with a protein whereas in the plant it is present as phosphoric esters the commonest being the hexaphosphate or phytic acid The mixed calcium magnesium hydrogen salt of this is known as phytin and it occurs in a number of cereals Phytic acid precipitates calcium ions to form the insoluble calcium salt the calcium and phosphorus of which cannot be utilized The phytic acid of cereals not only renders much of the calcium unavailable but also combines with the calcium of other foods e g milk and prevents its absorption Foods containing much phytic acid e g oatmeal are therefore rachitogenic (p 5_7) Phytic acid can also combine with iron to form an insoluble salt and so prevent its absorption from the gut

Inositol deficiency in mice and rats produces alopecia and loss of weight [138] Dogs kept on diets deficient in inositol and pantothenic acid show

increased gastric emptying time, pylorospasm, segmentation in the large and small intestine and gastro-intestinal hypertonicity and hypomotility [144]. Deficiency syndromes have also been produced in the guinea pig, pig and fowl.

The production of fatty livers in rats by feeding a beef liver fraction can be prevented by inositol, which has a lipotropic effect [484]. In pigs and rats the symptoms produced by administering sulphathalidine or sulphasuxidine can be relieved by inositol [252, 253].

The function of inositol is unknown. It is possible that it is not a prosthetic group of an essential enzyme system, like the other B vitamins, but an essential component of living tissue. It is uncertain if it is essential for human nutrition as no deficiency syndrome due to lack of the vitamin has been described. The probable daily intake in man is about 1 gram daily [339]. It is present in the blood to the extent of 0.37 to 0.76 mg per 100 ml [372] and about 12 mg are excreted in the urine in twenty-four hours [379]. It has been claimed that the administration of inositol to patients with gastro-intestinal cancer reduced the fatty infiltration of the liver [288]. Lupton and his co-workers [21] investigated the lipotropic effect of inositol in human atheromatosis, but they were unable to observe any beneficial effect after administering it for six months.

An antimetabolite for inositol is known. It is lindane ("gammexane"), the γ isomer of 1, 2, 3, 4, 5, 6-hexachlorocyclohexane, which is used as an insecticide. It may act by a process involving interference with the metabolism of inositol [348].

CHOLINE

$$(CH_3)_3 \equiv N(OH) \cdot CH_2CH_2OH$$

There has been some hesitation about accepting choline as a member of the vitamin B complex. Admittedly it is essential for nutrition, but it is required in such relatively large amounts (35 to 100 mg per kg body weight) that it is probably a structural component of the body tissues rather than a biochemical catalyst. Lack of it can produce a deficiency syndrome, but so can lack of protein or essential amino acids.

Choline in Foodstuffs

Food	Choline content in mg per gram
Asparagus	1.28
Barley	1.39
Bread, white	0.625
black	0.565
Brussels sprouts	1.03
Butter	0.4
Cabbage	2.51
Carrot	0.95
Cheese	0.48–0.56
Cod	2.0
Egg, white	negligible
yolk	17.13
Flour, white	0.52
Ham	0.88
Herring	1.27
Kidney, pig	2.56
lamb	3.6
beef	3.33
chicken	2.23
Leek	0.095

Choline in Foodstuffs—continued

Food		Choline content in mg per gram
Liver	pig	5.52
	beef	6.3
	calf	6.5
	chicken	3.42
Milk		0.147
Muscle	veal	0.7–1.4
	lamb	1.1
	pork	0.75–1.0
	beef	0.9
Oats		0.94
Onion		Nil
Parsley		0.16
Peas		2.63
Pork chops		0.77
Potato		1.06
Rice		1.07
Salmon		1.81
Soya bean		3.0
Spinach		2.38
Trout		0.87
Turnip		0.94
Wheat		0.92
	bran	1.43
	germ	4.1

Choline Deficiency The importance of choline as a food factor was first suggested several years ago by Best [31] who showed that in rats the addition of choline to a diet high in fat prevents the deposition of excess fat in the liver Choline is therefore said to be a lipotropic factor betaine the anhydride of choline and the amino acid methionine also appear to have a lipotropic action The exact mechanism of the lipotropic action of choline is unknown but it is assumed that dietary choline increases the phospholipid content of the liver and this promotes the transport of fatty acids as phospholipids from the liver to other tissues or promotes the utilization of fatty acids in the liver itself [30] A diffuse nodular cirrhosis of the liver occurs in a number of species kept on diets deficient in choline [235] and at the same time hæmorrhagic degeneration of the kidneys has been observed the lesion occurring in the cortex of the organ [32–34] A chronic choline deficiency produces destruction of the renal parenchyma in the rat [384] Hypertension also occurs in choline deficient rats [385] Not only is choline essential for the metabolism of neutral fat but also for that of cholesterol [238] Choline also prevents the formation of fatty livers in rats on diets deficient in aneurine This excess fat is due to synthesis from carbohydrate sources

Choline is able to prevent specific dietary hepatic injury in rats and protects against liver damage by toxins such as chloroform and carbon tetrachloride [25 36 383] A considerable reduction in phagocytic activity has been observed in rats fed diets deficient in choline [345]

Choline deficiency i
similarity between the c
in choline deficient ani
pointed out However
as lecithin of which choline is a component is widely distributed in animal and vegetable foods

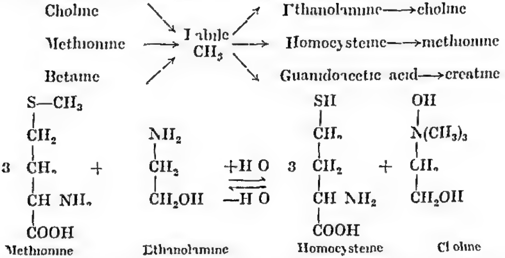
Lowry and his co workers [43] produced cirrhosis of the liver in rats resembling Laennec s cirrhosis in man Choline both prevented and cured

the condition although as would be expected the fibrous tissue persisted The liver cells regenerated and the gross appearance of the liver improved Similar findings are reported by Louts [226] Best and his co workers [386] noted that choline prevents liver damage in rats caused by feeding excessive quantities of sugar and alcohol Milk is not a very good source of choline (1 07 mg per gram) and Rao [229] has suggested that the infantile cirrhosis seen in Hindu children (a variety of portal cirrhosis) may be caused by a diet of cows milk and a *B coli* infection Recent clinical studies also point out the importance of a high protein diet in the treatment of patients with hepatic lesions

Functions of Choline Choline is essential for the normal nutrition of the chick and for egg production [232] for the prevention of perosis or slipped tendon in some birds [233] for the lactation and normal nutrition of the rat [235] Generally speaking the young growing animal needs more than the adult The choline requirement of the dog is about 35 mg per kilo of body weight daily [242] that of the chick is 75 mg daily [381]

In addition to the above mentioned functions choline is utilized in the animal organism for the formation of acetylcholine

The methyl groups of choline and the other lipotropic factors betaine and methionine play a part in the metabolic process known as transmethylation which is concerned with the shifting of specific methyl groups as such from one metabolite to another [382] The hypothesis has been advanced that methyl groups in a utilizable form are indispensable in the diet because the animal organism cannot itself generate the methyl groups for the essential methylations Thus methionine is essential for the growth of young rats Growth occurs however if they are fed a diet containing choline and homocysteine because a labile methyl group is supplied by the choline and donated to the homocysteine which is thereby converted to methionine Conversely choline can be formed by the methylation of ethanolamine methionine acting as a methyl donor [382] Another important physiological methylation is the conversion of guanidoacetic acid into creatine



Clinical Uses of Choline Very little choline is excreted in the urine or faeces The intake in the average human diet is about 650 mg daily It can to some extent be replaced by methionine

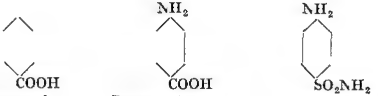
Choline has been used in the treatment of hepatic cirrhosis in man In 1937 Patek and Post [38 40] stated that a diet containing a large amount of the vitamin B complex had a favourable effect on the survival rate in patients with hepatic cirrhosis Since then several papers have appeared on the beneficial effects of choline on human hepatic cirrhosis particularly when associated with fatty infiltration [387 389] The results have not been conclusive because controlled studies are difficult to make The results in

advanced fibrotic cirrhosis have been disappointing The normal human diet contains a considerable quantity of choline and there is no evidence that a deficiency state due to choline deficiency exists in man Many workers have given doses as high as 6 grams a day which is approaching the toxic level the danger is a sudden flooding of the circulation with choline and consequent depression of the heart and blood pressure Herrmann and Rockwell [390] claim that the beneficial effects they observed might have been due to improved myocardial efficiency as the choline is converted to acetylcholine Choline and methionine have been used in the treatment of infectious hepatitis with conflicting results [391–394] Williams Cayer and Cornatzer [421] have shown that in patients with fatty infiltration of the liver the rate of p
taining radio
Moosnick
of choline chloride resulted in the hæmatological remission of a case of pernicious anæmia refractory to parenteral liver therapy Davis and Brown [396] failed to observe any beneficial effect in a case of pernicious anæmia that subsequently responded to parenteral liver therapy in one of nutritional megaloblastic anæmia in a case of megaloblastic anæmia of pregnancy and
ciated with the sprue syndrome
two cases resembling Addisonian
parenteral liver extracts Davis
direct erythropoietic activity but
that under certain circumstance it may potentiate the effect of liver extracts Watson and Castle [397] noted that choline increased the red cell and hæmoglobin values in a patient with hepatic cirrhosis and macrocytic anæmia

PARA AMINOBENZOIC ACID

There is some doubt about the status of para aminobenzoic acid (PABA) as a vitamin It is an integral part of one of the other B vitamins folic acid and is essential in animal and possibly in human nutrition although in the latter this has never been demonstrated It has not been conclusively shown that PABA is required nutritionally in addition to folic acid or that it has *catalytic functions independent of folic acid* PABA *is unique in that it is a vitamin within a vitamin* ' [398]

PABA was first synthesized in 1863 by Fischer although its claim to be a member of the vitamin B complex dates from only 1940 In that year Woods [39
yeast was
bacteria
of PABA



Benzoic acid Para aminobenzoic acid Sulphanilamide

In 1941 Ansbacher [401] stated that PABA is essential for the normal pigmentation of the rat and is a growth factor for the chick This was challenged by other workers but Martin [402] reconciled the opposing views by showing that PABA prevents achromotrichia only by altering the intestinal flora thus favourably influencing the bacterial synthesis of folic acid which is the factor preventing achromotrichia

The occurrence of PABA in foodstuffs is correlated with that of folic

acid, of which it is a constituent. Cereals contain from 0.3 to 1.0 micrograms per gram, vegetables from 0.18 to 0.6 micrograms per gram, ox liver 2.5 micrograms per gram; dried egg 0.2 to 0.36 micrograms and milk 0.15 micrograms per gram. Yeast is the richest source and contains from 4 to 100 micrograms per gram. Much of the PABA in foods is "bound" with proteins, amino acids or polypeptides.

Absorption and Excretion of PABA. PABA is absorbed rapidly from the *human intestine*, absorption being complete within eight hours, but no more than fifty per cent. of a dose of PABA given by mouth is absorbed [403]. By administering PABA containing the *radioactive isotope* N_{15} *Lustig*, Goldfarb and Gerstl [404] showed that there was no storage or utilization of the compound. Nineteen hours after injection only traces were detected in the organs, but eighty-two per cent. was found in the excreta. Most of the PABA excreted is in the conjugated form, i.e. acetylated as *p*-acetyl aminobenzoic acid. PABA is synthesized by the bacteria in the human gut because the fæcal excretion is considerably in excess of the dietary intake [405]. It is not known whether synthesized PABA is absorbed. It can be absorbed from the large intestine [403], and is probably absorbed from the bacteria that synthesize it. PABA is excreted in human sweat, which contains 0.0024 micrograms per gram [406].

PABA Deficiency in Animals. There is some doubt whether lack of PABA *per se* produces deficiency symptoms in animals. Those reported —grey hair in rats and mice [401], and failure of reproduction and lactation [407]—are probably indirect. Any action it may have is believed by most workers to be on the intestinal micro-organisms rather than on the animal. The quantities required to remove the effects of a so-called deficiency are far greater than those ingested in the animal's normal diet, or those that can be assimilated or utilized [408]. The small amount of PABA present in various animal tissues and fluids and finally excreted in the urine are probably derived from bacterial growth in the intestine and not from the food. Sulphaguanidine inhibits the growth of intestinal bacteria and if this is administered to human subjects there is a marked drop in the excretion of PABA, only traces being excreted [408]. It can therefore be considered that it originates in the intestine during the growth and multiplication of the bacterial flora. PABA is known to stimulate the growth of bacteria in the gut, and these bacteria produce several other vitamins, *in particular folic acid*. This can, in fact, cure deficiency symptoms said to be due to PABA [409].

Antibacterial Action of PABA. Although a growth factor for many micro-organisms, PABA in a concentration of 1 in 1,000 inhibits the growth of *M. tuberculosis*. The severity of infection with this organism is diminished and the survival time increased in guinea-pigs given 100 mg. of PABA daily [410]. On the other hand, the survival time of mice infected with typhoid is diminished by feeding PABA [410].

When PABA is incorporated in the diet to the extent of three per cent., it is remarkably effective in suppressing typhus infection in mice [411], and some rickettsial infections of chick embryos when injected into the yolk sac. This suggested its use in the treatment of some rickettsial infections in man (p. 142). The anti-bacterial and anti-rickettsial action of PABA can be inhibited by para-hydroxybenzoic acid [560],

Pharmacology and Toxicology. The LD_{50} by the oral route in mice, dogs and rats respectively is 2.85, 1.3 and 7.6 grams per kilogram [412]. Oral

doses much in excess of 1 gram/kg in dogs cause death following acute gastro enteritis and hæmorrhage into the small intestine

A number of toxic manifestations have occurred following the administration of PABA to human subjects Drug fever dermatitis medicamentosa nausea vomiting and leucopenia may occur after its administration [414] and myocardial renal and hepatic damage have been reported [559] Toxic hepatitis has been stated to occur after its administration [413]

PABA diminishes the toxicity of organic arsenicals such as acetarsone neoarsphenamine and tryparsamide [433] and also some organic bismuth preparations e g sodium bismuth tartrate [434] Clinically it does not prevent r … eactions or optic nerve injury produced … is not specific as it is produced by other … enylacetic and phenylpropionic acids [351]

The oral administration of PABA in man interrupts or greatly depresses the conjugation of ingested salicylate with glycine so that only very small quantities of salicyluric acid appear in the urine It also lowers the pH of the urine and thus decreases the renal clearance of free salicylate and it causes a decreased urinary excretion of salicylate and a rise in the plasma salicylate [415] It has therefore been proposed to administer PABA to increase the plasma salicylate without producing the symptoms of salicylism in the treatment of rheumatic fever and similar conditions without pushing the dose of salicylic acid From a clinical standpoint the combination of salicylate and PABA has little advantage over the administration of salicylates alone in the treatment of rheumatoid arthritis [482]

Clinical Uses of PABA Grey Hair and Skin Conditions After it was discovered that deficiency of PABA in the black or piebald rat resulted in greying of the fur (p 136) clinical interest centred around the compound in the hope that it might cure grey hair in humans A claim was made by Sieve [152] that it was effective for this purpose particularly in subjects with premature greyness but subsequent investigations by others did not confirm this [417 418] Recently however Zarafonetis [423] claims that massive doses of PABA e g 6 to 48 grams daily given for long periods do darken grey hair He recorded this during observations on the treatment of other conditions with PABA A possibility that cannot be excluded is the excretion into the hair of a p phenylenediamine like compound (which is a hair dye) derived from PABA and acting as a dye In this case the effect as in dyeing would not be permanent A warning is necessary against the continuous ingestion of PABA Although its acute toxicity is low it can cause leucopenia in man [414]

A claim has also been made that PABA in the form of its monoethanolamine derivative is effective in the treatment of vitiligo [353 373] This was based on its suspected ability to influence melanin formation Further investigations have failed to substantiate this [354]

It is stated that PABA protects the skin against sunburn and ointments containing it have therefore been used for this purpose [327] Rothman and Henningsen [422] using mercury arc lamps found that human skin with a layer of 0 03 mm of fifteen per cent PABA cream required fifty to a hundred times longer exposure to produce a threshold erythema than a … ehicle Shaw [424] has recommended … cent solution of PABA in seventy per … yer [425] have reported good results in … ABA in ten per cent alcoholic solution … Shaw claims that patients who were extremely sensitive to sunburn were able to expose themselves for eight to twelve hours to the Florida sun without discomfort PABA was however ineffective in photosensitization dermatitis

Zarafonetis and his co workers [426 429] describe the beneficial effect of

TREATMENT OF LUPUS ERYTHEMATOSUS WITH PABA

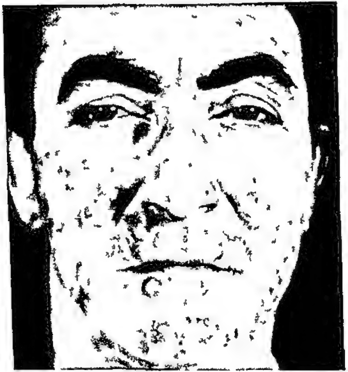


FIG 46 Patient with Lupus Erythematosus before treatment with PABA He had previously had treatment with bismuth gold and local therapy with little success

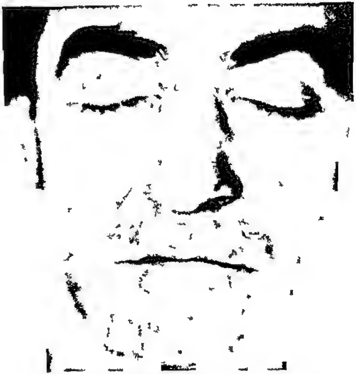


FIG 47 Patient with Lupus Erythematosus Same as in Fig 46 After five weeks treatment with PABA

TREATMENT OF LUPUS ERYTHEMATOSUS WITH PABA

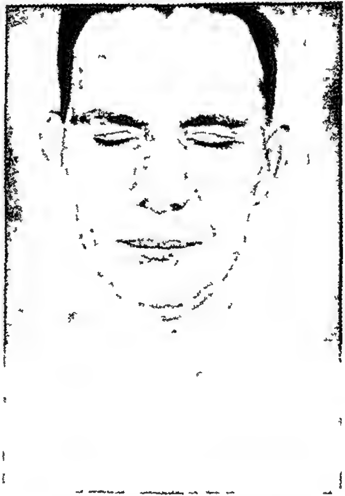


FIG. 18 Patient with Lupus Erythematosus before treatment with PABA

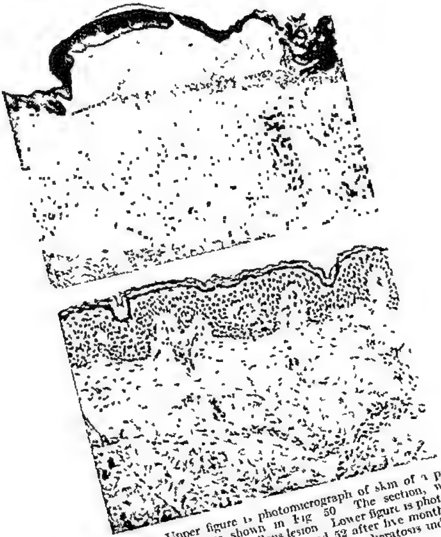
FIG. 19 The same patient as in Fig. 18 after ten weeks treatment with PABA

TREATMENT OF DERMATITIS HERPETIFORMIS WITH PABA

Fig. 50 Patient with Dermatitis Herpetiformis before treatment with PABA

Fig. 51 Same Patient with Dermatitis Herpetiformis as in Fig. 50 Five months after treatment with PABA

PABA or its sodium salt in the treatment of a number of skin conditions, including lymphoblastoma cutis, lupus erythematosus, scleroderma, pemphigus and dermatitis herpetiformis. All these disorders are of unknown pathology and the mode of action of PABA in their treatment is quite unknown. The PABA was administered in doses of 1 to 4 grams (10 to 40 ml. of ten per cent solution of the sodium or potassium salt) at intervals of two to three hours. Loewenthal [696] states that PABA is effective in a dosage of 1·5 to



Figs 52 and 53 Upper figure is photomicrograph of skin of a patient with dermatitis herpetiformis shown in Fig. 50. The section, which shows hyperkeratosis, is through a bullous lesion. Lower figure is photomicrograph of skin of same patient as in Figs. 50 and 52 after five months' treatment with PABA. There is only a slight degree of hyperkeratosis and lymphocytic infiltration and no bullæ.

4 grams four times daily in suppressing the manifestations of eczema and atopic dermatitis. Any effect produced is certainly a pharmacological one and not the result of remedying a deficiency.

Leukæmia. Following the observation that the administration of PABA to patients with rickettsial diseases (p. 142) causes leucopenia, it has been used in the treatment of leukæmia to reduce the white count. Zarafonetis and his co-workers [430] administered large doses of the sodium salt (2 to 4 grams every two hours day and night) to five patients with chronic myelogenous leukæmia; a striking lowering of the leucocyte count

In chicks folic acid is essential for the prevention of anæmia, for growth and for normal feathering. In its absence macrocytic hyperchromic anæmia, leucopenia and thrombocytopenia develop. Folic acid also prevents the perosis resulting from diets deficient in manganese, biotin and choline.

In the monkey folic acid deficiency is characterized by loss of weight, leucopenia, anæmia, hæmorrhagic diarrhœa, gingivitis, necrosis of the mouth and gums. The anæmia of folic acid deficiency in the monkey can be corrected with vitamin B_{12} and ascorbic acid, which may stimulate synthesis of folic acid in the body [698].

Folic acid cures the achromotrichia, anæmia, leucopenia and granulocytopenia caused by administering sulphonamides to rats. In the rat folic acid deficiency causes a pancytopenia and maturation arrest of the red and white blood cells at the level of the stem cell, progressing to total aplasia [701]. In mice folic acid appears to be necessary for lactation and for the maturation of immature blood cells.

Swine deficient in folic acid become anæmic, listless and and weak, their hair is shed and becomes lustreless and they suffer from diarrhœa, leucopenia and neutropenia [501].

The Significance of Folic Acid in Human Nutrition. The diet normally contains pteroylglutamic acid and/or its conjugates and the urine of both normal subjects and patients with pernicious anæmia contain it. It is not the extrinsic factor of Castle (which is vitamin B_{12}) because it cannot maintain patients with pernicious anæmia free from neurological complications [502]. It will convert a megaloblastic bone marrow to the normoblastic state but it cannot prevent the onset of the neural disturbances characteristic of untreated pernicious anæmia; it has in fact been said to accelerate their onset [502]. Megaloblastic anæmia is believed to occur if the diet is deficient in folic acid conjugates and vitamin B_{12}. Although folic acid produces an initial response in the treatment of megaloblastic anæmias both folic acid and vitamin B_{12} must probably be present in the body for normoblastic blood formation. If a test dose of pteroylglutamic acid is given pernicious anæmia patients they excrete less than do controls, suggesting that there is defective absorption, increased utilization or increased destruction of the compound in such patients [500]. Folic acid may be concerned with the oxidative metabolism of tyrosine [710].

Therapeutic Uses of Folic Acid. Folic acid produces a hæmopoietic response in many types of megaloblastic anæmia, including Addisonian pernicious anæmia [504] … pregnancy [521–523], the anæmia … nutritional anæmia [524–525], the … and that following gastrectomy [50… …cy in this country responds completely to folic acid and usually not at all to vitamin B_{12} [563]. Folic acid is ineffective in anæmias due to iron deficiency, the leukæmias, and those associated with hypoplasia or aplasia of the bone marrow. Although leucopenia and thrombopenia have been produced in animals deficient in folic acid, it has not proved successful in the treatment of patients suffering from these hæmatological conditions. The effective daily dose for optimal blood regeneration varies from patient to patient in the same way as experience has shown to be the case with liver extracts. Treatment is usually started with an oral dose of 10 to 20 mg. for fourteen days and if there is a satisfactory response the dose can be reduced to 10 mg. and finally to 5 mg. daily; a dose as low as 1 mg. may be effective in some cases [490], whereas some cases may require 20 mg. or more [504]. Large doses given parenterally or orally are unnecessary and wasteful unless the blood picture warrants it. In the majority of cases the *maintenance dose* is between 2.5 and 5 mg. daily.

Folic acid should never be given as maintenance therapy for the treatment of pernicious anæmia because it fails to prevent the onset of subacute combined

Fig 56 Patient with Sprue Three quarters of an hour after a barium meal showing a broken barium column isolated segments and stack of coins and wheel effects

Fig 57 Same patient as in Fig 56 one hour after a barium meal showing moulage of barium

FOLIC ACID IN THE TREATMENT OF SPRUE

Fig 58 Patient with Sprue The same as in Figs 56 and 57 after five weeks treatment with folic acid The alimentary tract shows normal radiological appearances

Fig 59 Same patient as in Fig 58 The barium meal has passed on further The radiological appearances are normal (Dr Spies case)

degeneration. The therapy of choice is liver or vitamin B_{12}. Before the introduction of vitamin B_{12} the use of folic acid was justified in patients sensitive to liver while they were being desensitized. There is no justification for its use in pernicious anæmia now. Although folic acid is at first effective in the treatment of pernicious anæmia, the anæmia tends to relapse and the dose has to be raised

In patients with sprue, idiopathic steatorrhœa and cœliac disease hæmato-ogical improvement usually occurs if the condition is associated with a megaloblastic anæmia. Evidence of the value of folic acid in improving the hæmatological and relieving the gastro intestinal symptoms of these conditions is, however, conflicting Spies and his colleagues [504–507] in the United States and Cuba report not only a striking hæmatological response but also the disappearance of the oral and gastro-intestinal symptoms in patients with tropical sprue treated with folic acid A remarkable improvement in the radiological appearances of the gastro intestinal tract was also described, including the disappearance of mucosal œdema and intestinal segmentation, spasm, dilatation and hypomotility (Figs 56 to 59) Darby, Jones and Johnson [508] also report a striking all round improvement in cases treated in the United States Darby and others [514] noted a return to normal glucose tolerance, improved vitamin A and tocopherol absorption, an increased prothrombin concentration and a decrease in fæcal fat On the other hand, Davidson and his co workers [508–510] in Edinburgh, whilst they confirm the dramatic control of diarrhœa and the rapid clinical improvement, consider the hæmatological response very disappointing They observed no beneficial effects from giving folic acid to patients with cœliac disease or idiopathic ulcerative colitis Weir and Comfort [512] of the Mayo Clinic also consider the response of non tropical sprue to treatment with folic acid disappointing, no general improvement occurred that could not be accounted for by improved diet and rest, and macrocytosis persisted Ferguson and Calder [512] record similar findings There was no evidence of improvement in fat absorption, biochemical findings, blood pressure or intestinal pattern in any patient with non tropical sprue treated with folic acid Whilst Fox [515] agrees that folic acid produces a beneficial effect in sprue, he states that it does not produce remission in all cases, and that it is inferior to liver extract in its effect on the blood picture

Evidence of the value of folic acid in the treatment of cœliac disease is slender Brody and Gore [516] and Thomson, Dalton and Wilson [517] noted rapid clinical and hæmatological improvement in three cases So did Tegelaers and Weyers [518], but their cases received yeast in addition Davidson and Girdwood [511] and Wilkinson [519] obtained an unsatisfactory response in their cases Carruthers [520] claimed to have obtained good results in six patients with severe recurrent chronic diarrhœa treated with folic acid; normal stools were obtained in two to five days The ætiology of the diarrhœa in these cases was obscure

Israels and Sharp [522] treated five patients suffering from idiopathic steatorrhœa (non-tropical sprue) with folic acid All patients responded promptly and fully to folic acid given by mouth or parenterally and reasonable blood counts were maintained, the patients were refractory to parenteral liver therapy

Granulocytopenia. Granulocytopenia produced in rats by feeding purified diets and sulphonamides or thiourea can be both prevented and cured by folic acid [526–528] Granulocytopenia and leukopenia produced by administering thiouracil and propylthiouracil to rats can also be prevented by the simultaneous administration of folic acid or liver [529]. Menten and Graff [530] have treated children suffering from granulocytopenia following the administration of sulphonamides with folic acid in high doses (150 mg daily) Thirteen out of twenty-two children showed a rise in the granulocyte to normal levels after seven to ten days, unfortunately no figures are given to

show the rate of recovery of those children whose sulphonamide was stopped and no other treatment given Black and Stanbury [537] and Waelsch [538] each described two cases of drug granulocytopenia that improved after treatment with folic acid, but spontaneous remission could not be excluded

Irradiation Sickness Goldfeder and his associates [531] state that folic acid protects mice against the fatal anæmia and leucopenia produced by X-radiation This has not been confirmed in the cat [532] or the pig [533] In man the evidence is conflicting Davis [534] reports that folic acid in doses of 75 to 150 mg three times daily diminished the depressant effects of radiation therapy on the bone marrow of sixty nine patients with lymphoblastoma treated with radiation therapy Jacobson [535], however, failed to observe any benefit in patients subjected to radiation after treatment with folic acid Cornatzer and his co workers [536] state that both folic acid and vitamin B_{12} have a protective effect on the hæmopoietic system of animals treated internally by radio active phosphorus, P_{32}

Folic Antagonists in the Treatment of Leukæmia and Malignant Disease Folic acid itself is without effect in the treatment of acute and chronic leukæmia [519] In 1944 Leuchtenberger and others [539] had found that a folic acid concentrate produced regression of sarcoma 180 transplanted into mice, although the work was not confirmed It was subsequently shown that the concentrate contained pteroyltriglutamic acid (teropterin), and pteroyl diglutamic acid (diopterin) These compounds have since been tried for the treatment of human malignant disease and allied conditions such as acute leukæmia and Hodgkin's disease Appetite and well being improve but there is little evidence that these folic acid derivatives have any effect on the disease process [540]

It has been found that pteroylglutamic acid or its conjugates intensify the leukæmic process in the leukæmias [541] Folic acid antagonists have therefore been tried for the treatment of these diseases The principal compounds that have been employed are 4 aminopteroylglutamic acid (aminopterin), 4 aminomethylpteroylglut
pteroylaspartic acid (amino an fol)
and also neoplastic growth [543,
leucocytes *in vitro* aminopterin produces marked inhibition of mitosis in low concentrations [544] The clinical use of such compounds is fraught with danger as they interfere with the nuclear mechanism and are toxic to normal as well as to malignant cells In minute doses, e g 0 003 mg, they inhibit the growth of the chick embryo [545] Their clinical use in the leukæmias dates from 1949, when it was hoped that in the folic acid antagonists one had a weapon for the effective treatment of this group of diseases Subsequent investigations [547–556] have shown that remissions do occur, but they are only temporary, often incomplete, and seldom of more than a few weeks' duration Folic antagonists are not curative in the leukæmias Improvement occurs more readily in children and rarely in adults, the
frequently than the myelo
arely occurs At best this
oblastic leukæmia a twenty
five per cent chance of a few weeks' remission It is necessary to supplement treatment with blood transfusions and antibiotics Toxic reactions which occur in nearly every case include a greatly increased tendency to bleeding (this may be fatal), stomatis diarrhœa, vomiting and alopecia In addition to hæmorrhage into the skin and mucosæ and bleeding from mucosal surfaces hæmorrhage into the lungs and middle ears may occur These hæmorrhagic complications have been responsible for many deaths Although the results obtained with folic acid antagonists in the treatment of the leukæmias are disappointing, they are of considerable theoretical interest and may furnish a lead in the discovery of a new series of drugs of real value in the leukæmias and malignant disease

Folic Antagonists in the Treatment of Arthritis and Psoriasis Aminopterin is a potent inhibitor of connective tissue proliferation [557] Administration of the folic acid antagonists to patients with rheumatoid arthritis results in a remission of symptoms and signs of articular and periarticular inflammatory action [558] This is understandable as this form of arthritis is characterized by very pronounced connective tissue proliferation Aminopterin has no effect on the disease process since the sedimentation rate and gamma globulin are unchanged The effect of aminopterin in rheumatoid arthritis is not mediated through the adrenal cortex like cortisone or ACTH but appears to be due to inhibition of mesenchyme The effect of aminopterin is not confined to mesenchymal derivatives Striking remissions of the lesions of psoriasis lupus erythematosus and atopic eczematoid dermatitis have been reported [558] The toxic effects of aminopterin limit its use as a practical therapeutic agent in these diseases

THE VITAMIN B_{12} GROUP

History The history of vitamin B_{12} begins with the discovery of Minot and Murphy in 1926 that liver is curative in pernicious anæmia Various active concentrates were obtained from liver from time to time but progress was hampered by the lack of a suitable assay technique all assays having to be made on pernicious anæmia patients in relapse It was the introduction of a microbiological method of assay of the hæmopoietic principle of liver that speeded up the testing of highly active liver concentrates In 1947 Shorb [564] observed that there is a linear relation between the content of the *Lactobacillus lactis* Dorner growth factor of liver extracts and their potency in curing pernicious anæmia patients in relapse In the following year Rickes and his co workers [565] in America and Lester Smith [566] in England independently announced the isolation of the anti pernicious anæmia factor in pure crystalline form from liver concentrates It was called vitamin B_{12} and later cyanocobalamine Particular credit must be given to Lester Smith because he was unaware of the work of Shorb and all his preparations had to be assayed clinically Clinical studies by West [567] and Ungley [568] showed that this crystalline substance was not only active in pernicious anæmia in relapse but was also effective in the treatment of subacute combined degeneration associated with pernicious anæmia The daily clinical requirement was of the order of only 0 5 to 1 0 microgram This effective concentration is so small that it has been suggested that a single molecule is concerned in reaction with each of the red blood cells

Several derivatives of vitamin B_{12} have been prepared e g vitamin B_{12a} vitamin B_{12b} vitamin B_{12} and vitamin B_{12d} [569 590] The *a* *b* and *d* compounds are probably identical [731] Vitamin B_{12} contains a nitrite group co ordinated with cobalt [590] Another form of vitamin B_{12} has been obtained from rat fæces [718]

There is now little doubt that the extrinsic factor of Castle is vitamin B_{12} itself When administered orally in pernicious anæmia the pure vitamin is ineffective except in very large doses but small doses are strongly potentiated by normal gastric juice [572]

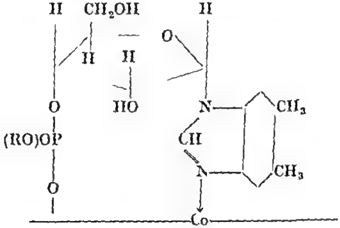
Chemistry The chemistry of vitamin B_{12} is reviewed by Lester Smith [729] It was originally isolated from liver After Rickes and his group [573] isolated it from the metabolism fluid of *Streptomyces griseus* it was produced on a commercial scale as a by product in the manufacture of streptomycin This has resulted in a considerable lowering of manufacturing costs The original yield from liver was only 15 mg from 1 000 kg of liver A crystalline product similar to vitamin B_{12} has been obtained from cultures of *Streptomyces aureofaciens* which is used in the production of aureomycin [563]

Vitamin B_{12} crystallizes in small dark red needles Shortly after

discovery it was shown that cobalt in the form of a co-ordination complex was an essential constituent of the molecule [574]. It also contains a cyanide group co-ordinated with the cobalt atom and for this reason it has been given the name cyanocobalamine in the British Pharmacopœia. Vitamin B_{12b} differs only in the absence of this cyanide group and is readily converted into

FIG. 60. Crystals of Vitamin B_{12} obtained from *Streptomyces griseus* cultures

vitamin B_{12} by treatment with potassium cyanide [575]. The vitamin B_{12} molecule contains a 5 : 6-dimethylbenziminazole residue [576], phosphoric acid, ribose, 1-amino-2-propanol [577] and a cobalt-containing acid with the formula $C_{43}H_{33}O_4N_9Co$. The probable molecular weight is 1,300. It probably contains the following structure with the cobalt in the form of a co-ordination compound.



Vitamin B_{12} appears to be stable in the solid state and in solution at neutral or slightly acid pH values. It is slowly inactivated in solutions of strong acids or alkalis, but withstands autoclaving for fifteen minutes at 121° C. If kept under sterile conditions in isotonic solution it can be stored at room temperature for more than a year without loss of activity.

Assay of Vitamin B_{12}. The original assay method of Shorb [564] using the growth of *L. lactis* Dorner has not given satisfactory results in the hands of other workers. The addition of tomato juice and Tween 80 is said to be

necessary in the growth medium [578] More satisfactory results are said to be obtained using *Lactobacillus leichmannii* as the test organism in conjunction with paper chromatography, the paper strips either being laid on agar plates on which the bacillus has been sown or cut into strips each strip being assayed separately [579–581] The alga *Euglena gracilis* has also been used [586]

Animal assays have also been used One is based on the increase in weight of chicks from hens deficient in vitamin B_{12} [582] another is based on the growth of vitamin B_{12} depleted rats [583] A colorimetric assay method has been devised, depending on the formation of a blue to purple product after acid hydrolysis and its extraction with a mixture of light petroleum and *n* octyl alcohol [584] Another chemical method depends upon the liberation of cyanide on exposure to light [571]

Units The amount of vitamin B_{12} is expressed in weight (micrograms)

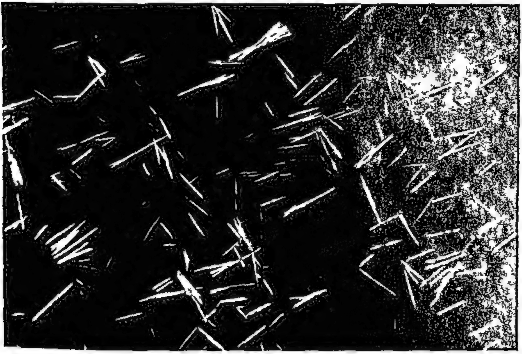


FIG 61 Crystals of Vitamin B_{12} obtained from Liver

of active substance One microgram of the pure vitamin is stated to have the activity of 1 U S P unit of anti pernicious anæmia activity although this may be an overestimate according to Jones Darby and Totter [591] it is only a half to a third as potent as this

Occurrence of Vitamin B_{12} In view of the doubtful specificity of the methods of assaying the vitamin B_{12} group figures giving their distribution in foods must be accepted as provisional only The richest known sources are liver kidney and the nutrient medium in which *Streptomyces griseus* is grown Meat, milk cheese eggs and fish are good sources while cereals and yeast are poor sources [585] The tissue fluid of fish contains a cobalt pigment which yields a hæmopoietically active substance on treatment with potassium cyanide [717] Vitamin B_{12} is also present in animal dung muscle and bacter[illegible] B_{12} content of some foods is on p 156

[illegible] min B_{12} is as effective as liver in con

tr[illegible] ernicious anæmia Not only does it

cc[illegible] to a normoblastic type [591] but it also prevents the onset of subacute combined degeneration of the cord [592]

	Micrograms per 100 gram Rat growth	L. leichmannii
Alfalfa meal	—	4
Beef	2–5	4
Cheese	—	—
Dried milk	2–3	—
Egg yolk	2–8	—
Fish meal	—	9–10
Herring	15	—
Kidney	35–50	—
Liver	35–50	—
Liver extract	—	20–2,200
Milk	—	9–10
Mutton	5	4
Pork	1–3	4
Soya bean	—	1
Veal	4	4
Wheat	—	0 7

and relieves the symptoms of glossitis [593] Vitamin B_{12} is active parenterally and also orally in much larger doses (thirty to eighty times), or when administered with gastric juice or some other known source of intrinsic factor [594] Five hundred ml of normal gastric juice is probably needed to ensure the adequate absorption of 50 micrograms of vitamin B_{12} The effectiveness of liver and hog stomach preparations when given orally in pernicious anæmia may be explained by their content of vitamin B_{12} and intrinsic factor Ternberg and Eakin [596] have shown that the non dialysable, heat labile component of normal gastric juice unites with vitamin B_{12}, it is less abundant than normal in the gastric juice of pernicious patients, and may be Castle's intrinsic factor. A simple view of the ætiology of pernicious anæmia would be that, as a consequence of atrophic gastritis the intrinsic factor is produced in sufficient quantity and that the vitamin B_{12} of the food fails to be modified by it and therefore absorbed There is experimental evidence to support the view that the intrinsic factor promotes the absorption of vitamin B_{12} by forming a readily absorbed peptide complex [674, 720] The fæces of pernicious anæmia patients in relapse contain large quantities of vitamin B_{12}, which is apparently not available [600], although extracts of these fæces are active if injected into other patients with untreated pernicious anæmia

Vitamin B_{12} can be utilized locally by the bone marrow cells without any change by intrinsic, or any other factors, which presumably therefore aid its absorption Direct instillation of the vitamin into the bone marrow cavity of a patient with pernicious anæmia in relapse corrects a qualitative abnormality in cellular ribonucleic acid [596] Folic acid is not used locally by bone marrow cells within forty-eight hours, it is probable that it must first be converted into an active hæmopoietic substance by enzyme activity elsewhere in the body Both folic acid and vitamin B_{12} are probably involved in chemical reactions leading to the formation of nucleic acids in the living cell [597, 742] Vitamin B_{12} appears to be necessary for the formation of ribosides, such as thymidine, from purines and pyrimidines Thymidine replaces vitamin B_{12} for the growth of *L lactis* [597] and in large amounts for *L leichmannii* [598], but it has no erythropoietic effects in patients with pernicious anæmia [599]

In the mammal a deficiency of vitamin B_{12} has a deleterious effect on growth [603] even if the protein intake is high, and it has been suggested that the vitamin plays a fundamental role affecting the capacity of the normal mammal to utilize protein [601] According to Henry and Kon [602] this effect may not be specific, aureomycin can replace vitamin B_{12} as a growth factor in the rat [690] Pigs fed 10 micrograms of vitamin B_{12} daily

show a g ... ntrols not receiving the vitamin [604] (... l as by products in the manufacture (... feed supplements for livestock A deficiency syndrome characterized by failure to gain weight has been produced in the human infant fed on a purified ration [605] The factor responsible is soluble and present in milk, it may be vitamin B_{12} A cautious attitude must, however, be assumed to the use of vitamin B_{12} to augment the growth of healthy infants [606] The vitamin is essential for reproduction and lactation in the rat [687] In the chick it spares pantothenic acid [725]

There is some evidence that vitamin B_{12} plays a role in the metabolism of the amino acids methionine and tyrosine It protects weanling rats against kidney hæmorrhage induced by diets low in methionine and choline [607], and has a lipotropic effect in rats receiving a diet low in protein and fat, and in choline and methionine [608] It is choline sparing in the rat [722] and appears to be essential for the utilization of methionine or betaine in the biosynthesis of choline [722] The vitamin is probably necessary for the transformation of homocystine into methionine [609, 610] and glycine to serine [744] Vitamin B_{12} may function in enzyme systems involved in the synthesis and utilization of the labile methyl group and in the process of transmethylation, e g the transformation of glycine to choline and homocystine to methionine [611] The concept of transmethylation supposes the need for preformed methyl groups in the diet which can be transferred from one compound to another to form essential metabolites from precursors existing in the diet or the body (see p 134) A defect in methylation is seen in dogs deficient in vitamin B_{12} and results in fatty degeneration of the liver [726]

An aberrant tyrosine metabolism occurs in untreated pernicious anæmia, in which the blood phenol level is high and the urinary hydroxyl phenolic acids are increased The administration of vitamin B_{12} to pernicious anæmia patients causes a fall in the excretion of these acids [612]

In the animal vitamin B_{12} protects against the toxic effects of thyroxine [613] Wayne and his co-workers [614] conclude that in doses up to 100 micrograms it has no significant effect on human thyroid function

Absorption, Storage and Excretion of Vitamin B_{12} Vitamin B_{12} is poorly absorbed from the gastro intestinal tract In man the major portion is excreted in the fæces and practically none in the urine which appears to have little vitamin B_{12} activity when tested microbiologically [615] The daily excretion in normal subjects is about 0 15 micrograms and about a third of this in pernicious anæmia patients in relapse [745] Even when massive doses e g 10 000 micrograms, are given orally to normal subjects or pernicious anæmia patients practically none is excreted in the urine [688] The vitamin is synthesized by bacteria in the gut, the administration of cobalt and aureomycin increase the amount the latter acting presumably on bacteria that compete with the vitamin synthesizing bacteria [686] 1 2 dichloro 5 diaminobenzene, inhibits the bacterial synthesis of vitamin B_{12} probably blocking the utilization of an essential metabolite 1 2 dimethyl 4 5 diaminobenzene, which forms a part of the vitamin B_{12} molecule, on the other hand, stimulates bacterial synthesis [682] 1 2-dichloro 4 5 diaminobenzene is an antimetabolite of 1 2 dimethyl 4 5-diaminobenzene [683] In the rat given vitamin B_{12} containing radioactive cobalt about five per cent appears in the urine over a period of three days, most being excreted in the first day [616] Small amounts of vitamin B_{12} given by mouth are only effective in man if accompanied by a source of intrinsic factor [572] Ungley [617] has made a careful study of the absorption of vitamin B_{12} in pernicious anæmia patients He has shown that an oral dose twenty to forty times that effective parenterally is needed for an adequate response, Spies [619] gives a factor of 50 and other authorities

give the figure 200 [688] According to Ungley some vitamin B_{12} can apparently be absorbed without combining with the intrinsic factor At least 100 ml of *normal* gastric juice is needed to ensure an adequate response from 1 to 2 micrograms of vitamin B_{12} given orally the equivalent volume of pernicious anæmia gastric juice would be enormous and certainly beyond the capacity of the atrophic stomach of the pernicious anæmia patient Doses of 3 000 micrograms are effective given by mouth alone so that some must be absorbed Ungley showed that [illegible] buccal mucosa or directly from the [illegible] intrinsic factor probably does not [illegible] destruction by bacteria in the gastro intestinal tract because absorption is not facilitated by sterilizing the latter with antibiotics If vitamin B_{12} is injected into the human subject ninety to ninety five per cent of the dose promptly appears in the urine [688] half *is excreted within three to five* hours and most of it within twelve hours The figures are the same for normal subjects and pernicious anæmia patients It is therefore certain that the fate of orally *administered vitamin B_{12} differs from that of the* injected vitamin The failure to appear in the urine when given orally suggests that it is largely unabsorbed or that after absorption it is combined *in such a form that the kidney cannot excrete it (Ungley)*

The evidence to date suggests that failure of absorption of vitamin B_{12} occurs in pernicious anæmia rather than its destruction in the intestine or *its preferential utilization by the bacterial flora of the gut Chronic intestinal* disorders may lead to impaired absorption of vitamin B_{12} and so result in macrocytic anæmia

The vitamin B_{12} content of human blood is negligible [624] according to Conley and his co workers it is less than 3 5 millimicrograms [688] and according to others [745] it is 100 to 720 millimicrograms The level is lower *in patients with pernicious anæmia in relapse [745] Eight hours after the* intramuscular injection of a solution containing 1 mg of vitamin B_{12} none is found in the blood most is found in the urine [620] Fifteen minutes after the injection of 1 mg about *30 micrograms of vitamin B_{12} is found in a milli* litre of whole blood and 50 to 80 micrograms in a millilitre of plasma After the intramuscular injection of doses from 20 to 75 micrograms from seventy to ninety per cent *is retained in the tissues the bulk of the remainder is* excreted within eight hours [743] Others have reported an excretion of fifty three to sixty eight per cent after parenteral doses of 84 to 210 micro grams [746] *The vitamin does not diffuse into the red cells [615]* About 0 5 per cent of an oral dose of 0 89 mg is found in the liver and kidneys The amounts found in other organs are negligible [616 621] The daily excretion in the stools is of the order of 5 micrograms [621]

Toxicology Vitamin B_{12} is virtually non toxic even in a dose ten million times the therapeutic one Mice tolerate 1 600 mg per kg without any ill effects [676]

Requirements The vitamin B_{12} requirements of the healthy individual are quite unknown The daily intake in the food may be of the order of *2 to 10 micrograms daily depending on the diet* A daily intake of 1 micro gram provided it is absorbed is probably sufficient to maintain a normal blood picture Considerable vitamin B_{12} is synthesized by the bacterial *flora of the lower bowel* it is not known whether an exogenous source is necessary The fæces of pernicious anæmia patients have vitamin B_{12} activity [600 622] and an extract from them produces a hæmopoietic *response if injected* into another pernicious anæmia patient [623]

Clinical Use of Vitamin B_{12} Generally speaking vitamin B_{12} is efficacious in those forn[illegible] that respond to injections *of refined* liver extract [illegible] vitamin has not been reported it can occur [illegible] ade from liver or streptomycin culture broth [625] Reactions are more likely to occur in subjects sensitized

to antibiotics prepared from moulds [728] The clinical aspects of the subject up to 1951 are well reviewed by Ungley [730]

Pernicious Anæmia Numerous accounts have now been published of the efficacy of vitamin B_{12} in the treatment of pernicious anæmia following the original reports of West [567] Spies [587] and Ungley [568] It is effective in pernicious anæmia patients with or without subacute combined degeneration of the cord Probably 1 microgram daily is sufficient for maintenance In uncomplicated cases the initial dose should be 15 to 20 micrograms parenterally once or twice a week that is about 3 micrograms daily until remission occurs then a maintenance dose equivalent to 1 mg daily i e 15 mg every other week Ungley [627] recommends 40 to 80 micrograms initially and then 20 micrograms weekly for three months and 30 to 60 micrograms every three weeks thereafter Much larger doses have been given by mouth although thirty to sixty times the parenteral dose is needed for a response In pernicious anæmia with subacute combined degeneration of the cord 15 to 30 micrograms parenterally once or twice a week should be given until remission occurs then a maintenance dose of 15 micrograms every other week An initial dose of 200 to 300 micrograms parenterally in pernicious anæmia is suggested by some workers

The Sprue Syndrome Spies and his co workers [628] in the States have treated a number of cases of tropical sprue with vitamin B_{12} They report a prompt clinical and hæmatological improvement characterized by an increase in the reticulocytes a subsequent rise in the blood cells and hæmoglobin *and considerable improvement in the gastro intestinal symptoms* and radiological appearances Fifteen to 30 micrograms parenterally once or twice a week is stated to induce remission and 15 micrograms weekly thereafter to prevent relapse It is also active sublingually [748] The results of Spies and his co workers cannot necessarily be applied to tropical sprue as known to most British workers their cases were taken from a very under nourished population No well documented British reports are available at the moment Folic acid is stated to potentiate the hæmopoietic effect of

loses of 1 67 mg of folic acid and 25 micro

optimal response in sprue [737] Given by

micrograms of vitamin B_{12} daily for two to three weeks or more are necessary to produce a clinical response [741] Tuck and Whittaker [629] and Israels and Sharp [630] failed to observe any response to vitamin B_{12} therapy in megaloblastic anæmia associated with idiopathic steatorrhœa Cooke Peeney and Hawkins [631] obtained varied hæmatological responses in the latter condition treated with vitamin B_{12}

Tropical Nutritional Anæmia Spies and his colleagues [618] and Jones Darby and Trotter [591] obtained a hæmatological response in cases of nutritional macrocytic anæmia treated with vitamin B_{12} in America and Cuba and Patel [632] reported satisfactory results in India A single dose of 15 mg is said to produce a favourable initial response but it may be necessary to repeat this at two week intervals to prevent relapse It should be pointed out that nutritional anæmias in different parts of the world may present different hæmatological pictures and the ætiology may be due to a combination of many different factors such as malnutrition infection and infestation with helminths and parasites Vitamin B_{12} is ineffective in the treatment of anæmia due to *Diphyllobothrium latum* (fish tapeworm) [685] probably because the tapeworm absorbs the vitamin

Megaloblastic Anæmia of Pregnancy and the Puerperium Although the megaloblastic anæmia of pregnancy and the puerperium respond dramatically to the administration of folic acid according to most workers vitamin B_{12} is ineffective in treatment [633 637]

Megaloblastic Anæmia of Infancy Infants fed on proprietary brands of milk powder sometimes develop this anæmia which is rare in this country but has been reported in the United States It responds to folic acid (p 148)

but its response to vitamin B_{12} is variable [638 640] The effectiveness of vitamin B_{12} is possibly enhanced by ascorbic acid [640]

Megaloblastic Anæmia after Gastrectomy The megaloblastic anæmia that sometimes follows total gastrectomy is stated to respond to vitamin B_{12} [641] The clinical response is apparently good but the hæmatological response is suboptimal compared with that expected in pernicious anæmia The macrocytic anæmia of rats produced experimentally by operations on the bowel respond to treatment with vitamin B_{12} [712]

Leucopenia Vitamin B_{12} has no effect on the leucopenia induced by X radiation [646]

Neurological Conditions Vitamin B_{12} is effective in controlling the lesions of the central nervous system that result from the continued treatment of pernicious anæmia with folic acid [642]

Bean Franklin and Salis [643] state that painful nutritional neuritis is promptly relieved by the injection of 15 micrograms of vitamin B_{12} They do not consider the results due to analgesia *Spies and Stone* [644] *treated* five cases of amyotrophic lateral sclerosis with vitamin B_{12} Stiffness and muscle cramps disappeared and the patients felt stronger The neurological lesions associated with diabetes are stated to be relieved by *doses of 15 to 30* micrograms daily reduced to once or twice weekly [721]

ed sclerosis [645]

amin B_{12} stimu

late owth of children was studied Wetzel and his colleagues [647] considered that the administration of 10 micrograms daily produced a statistically significant growth response and increased physical vigour and appetite Chow [615] states *that vitamin B_{12} given to both ill and healthy children produces a gain* in weight greater than that of controls Rascoff and his co workers [689] observed no significant gain in weight in normal and premature infants given vitamin B_{12} Downing [679] *noted no beneficial effect in premature infants* and Chinnock and Rosenberg [706] none in normal infants The Council of Foods and Nutrition of the American Medical Association [606] adopts a sceptical attitude towards the results of these experiments and warns that the *conclusions must be accepted with reserve*

The Animal Protein Factor For many years it has been known that a water soluble factor known as the *animal protein factor (APF) is required for the survival and early growth of chicks and for the hatchability of eggs It is present in cow manure hen fæces* fish meal and the alcohol soluble fraction of liver Subsequently it was shown that APF is required for the growth of the mouse It may be transmitted from the mother to the young during gestation and lactation It was considered that APF is the same as vitamin B_{12} since it is active in the treatment of pernicious anæmia [669 670] The two are not identical For example *APF can be* replaced by a mixture of vitamin B_{12} and aureomycin or other antibiotics [680] Stokstad and his co workers [669] found that a *micro organism* from hen s fæces could produce APF in suitable media and that concentrates of *the APF produced a hæmopoietic response* in pernicious anæmia Vitamin B_{12} can replace APF as a growth factor for chicks [671] and APF will give a growth response resembling that of vitamin B_{12} with *Lactobacillus leichmannii* [672] APF is effective in the treatment of the megaloblastic anæmia of pregnancy [727] In later investigations Stokstad and his co workers [673] considered that APF is vitamin B_{12} and another *unidentified* factor

THE CITROVORUM FACTOR (FOLINIC ACID LEUCOVORIN)

In 1948 Sauberlich and Baumann [648] showed that liver liver extracts yeast extracts and commercial preparations used in the treatment of per

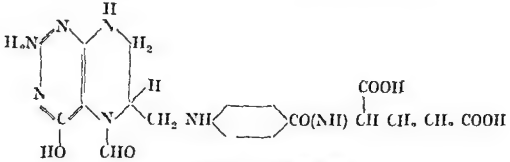
nicious anæmia contain a growth factor for the organism *Leuconostoc citrovorum*. This factor is not folic acid because it will promote the growth of some bacteria while folic acid will not, and it is not vitamin B_{12} because it can be separated from the latter by electrolysis [649]. Sauberlich and Baumann [650] later prepared a concentrate of the citrovorum factor that was much more active than folic acid, and Sauberlich [651] showed that in animals and man the urinary excretion of the factor varied with the folic acid intake. Intestinal synthesis is probably not a factor in the production of the citrovorum factor because the urinary excretion is not diminished by suppressing the growth of the intestinal bacteria with sulphonamides [651]. These observations suggested that folic acid might be converted into the citrovorum factor, or else stimulate its excretion. Nichol and Welch [652] showed that liver slices from folic acid deficient rats synthesized the citrovorum factor if incubated with folic acid in the presence of ascorbic acid,

FIG. 62. Crystals of the Calcium Salt of Leucovorin (Folinic Acid, the Citrovorum Factor)

thus proving that folic acid is essential for the formation of the factor. Vitamin B_{12} and ascorbic acid probably play some part in the conversion of folic acid into the citrovorum factor [684, 709]. The observations of Spray and Witts [784] suggest that in pernicious anæmia there may be a defect in the conversion of folic acid to the citrovorum factor. Cortisone can replace citrovorum factor in the growth of *L. citrovorum* [715].

Another group of workers had meanwhile shown that crude liver extracts are many times more active than folic acid itself in inhibiting the action of folic acid antagonists such as methylfolic acid [653]. From these liver extracts a potent concentrate was obtained and from it was isolated a substance which, on the basis of structure and functional relationship to folic acid, was called folinic acid. Analyses revealed the presence of at least two other factors of what was termed the folinic acid group. Further biological and chemical studies suggested the identity of the citrovorum factor and folinic acid. The synergistic action of folinic acid and thymidine (p. 145) was observed by Bond and his colleagues [653], who considered that it might be

involved in the synthesis of thymidine The same group later reported the synthesis of a substance, similar if not identical with folinic acid by reducing formylfolic acid in the presence of ascorbic acid [654] This was shortly followed by the synthesis and isolation in crystalline form by Brockman and his co workers [655] of a compound which appears to be identical biologically with the citrovorum factor [716] It is formyl tetrahydrofolic acid [707] also known as leucovorin



Folinic acid or leucovorin

It is unstable in the region of pH2 [714]

Vitamin B_{12}, Folic Acid, Thymidine, the Citrovorum Factor and Nucleic Acid Synthesis It is believed that vitamin B_{12} folic acid thymidine and the citrovorum factor all function in the biosynthesis of the nucleic acids The latter are essential for the synthesis of nucleoproteins and red and white blood cells The primary defect in pernicious and related anemias may well be the inability to synthesize certain nucleosides (particularly thymidine) which are integral parts of the nucleic acid molecule, from parent purines and pyrimidines

Nucleic acid

Nucleotide — Desoxyribose — H_3PO_4 — Adenine or Guanine (Purines) or Cytosine or Thymine (Pyrimidines)

Nucleoside e g Thymidine (=desoxyribose+thymine)

Nucleoproteins

↑

Nucleic acid

↑

Nucleotides

↑ H_3PO_4

Precursors ⟶ Desoxyribosides ⇄ Thymidine Citrovorum factor

(A) (B)

Jukes Broquist and Stokstad [656] have shown that *Lactobacillus leichmannii* can carry out step B but not step A and conversely *Leuconostoc citrovorum* can carry out step A but not step B They found that on purified media *L citrovorum* had vitamin B_{12} activity while *L leichmannii* had citrovorum factor activity Folic acid and the citrovorum factor

may be concerned with the "shuttling" of a formyl group in the formation of a pyrimidine (thymine) and its derivative (thymidine), the formation of purine derivatives, and the utilization of glycine in a number of reactions. The ability of mice to incorporate formate containing radio-active carbon (C^{14}) into the purines of nucleic acid is profoundly depressed by the administration of aminopterin, a folic acid antagonist [711].

The citrovorum factor is lipotropic and choline-sparing and is essential for the utilization of methionine and betaine in the biological synthesis of choline [722].

Hæmopoietic Activity of Citrovorum Factor. May, Sundberg and Schaar [657] treated megaloblastic anæmia of dietary origin in scorbutic monkeys with crude folinic acid. Fifty micrograms produced a prompt response comparable with that produced by 15 mg. of folic acid. Spies and his colleagues [658] and others [708, 723, 747] have shown that in adequate amounts the citrovorum factor is an effective hæmopoietic substance in patients with pernicious anæmia, nutritional macrocytic anæmia and tropical sprue in

[illegible]

Five to 15 mg daily intramuscularly is effective in pernicious anæmia according to Hausmann and Mulli [733]. This is to be contrasted with the dose of vitamin B_{12}, which is a thousand times less.

Citrovorum Factor and Folic Acid Deficiency. The citrovorum factor is more effective than folic acid in reversing a condition of folic acid deficiency in animals produced by aminopterin (p. 145) or by sulpha drugs [661]. It is of interest that aureomycin also overcomes the toxic effects of aminopterin. Clinically, synthetic citrovorum factor is effective in reversing the toxic manifestations of amethopterin and aminopterin, folic acid antagonists used for the treatment of leukæmia and other neoplastic diseases [662, 719]. Schoenbach, Greenspan and Colsky [662] treated two patients with leucopenia and ulcerative mouth lesions following therapy with amethopterin and aminopterin for metastatic neoplasms. The lesions healed and the leucopenia disappeared although previous treatment with high doses of folic acid had been ineffective.

Vitamin B_{13} and Vitamin B_{14}

Vitamin B_{13} is an unidentified growth factor for rats isolated from distiller's dried solubles by Novak and Hauge [663] in 1948. It may be identical with the animal protein factor (p. 160) or vitamin B_{12}.

Vitamin B_{14} is a crystalline compound isolated from urine that is extremely active in stimulating the proliferation of new cells in bone marrow cultures [664]. It is effective in curing experimentally induced anæmia in rats and is inhibited by folic acid antagonists such as methyl folic acid. Enzymes from milk, liver and gastric juice act on xanthopterin, folic acid and pteroyl triglutamic acid (teropterin) to form vitamin B_{14}, or at any rate related substances with identical activity [665].

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CHAPTER III

ANEURINE

(VITAMIN B_1, THIAMIN)

HISTORY

MODERN beriberi, a disease partly due to aneurine deficiency, dates from the introduction of steam-powered rice mills in the nineteenth century. When beriberi became prevalent in the East it was confined to those persons whose diets consisted largely of highly milled or polished rice from steam mills. In the same regions, those natives who ate rice ground in primitive mills largely escaped. The conquest of beriberi started from 1882, when Takaki [5], the Director-General of the Medical Department of the Japanese

ease among Japanese sailors by supple-

ainly of rice, with fish, vegetables, meat

deal with the disease in the Dutch East

Indies, where Eijkman [6] showed in 1897 that a paralytic condition in fowls, *polyneuritis gallinarum*, which closely resembled beriberi in its polyneuritic symptoms, could be produced by a diet consisting of polished rice. When the fowls were fed on unpolished rice they did not develop the disease, which was cured in afflicted birds by the administration of rice polishings. Grijns [7], Eijkman's successor in the East Indies, concluded in 1901 that both beriberi and avian polyneuritis resulted from the lack of a certain dietary factor or factors present in rice bran. His work focussed attention on beriberi as a deficiency disease, but this view was not seriously considered until Fletcher [8], Fraser and Stanton [9] confirmed it by their studies on beriberi in the Malay States between 1905 and 1910. Fletcher, working in an asylum at Kuala Lumpur, separated the patients into two groups, one of which was supplied with polished ric ... group thirty-six out of 120 deve ... ease, in the second group only ... were no deaths. Fraser and Stanton took 300 healthy labourers into a railroad labour camp. To half of them the customary polished rice was given as a staple article of diet, the rest receiving the unpolished grain. In three months time beriberi was rife amongst the white rice group, while those on unpolished rice were practically free from the disease. Later the rice rations were reversed in the two groups, with the result that the disease disappeared in the first group and an epidemic of beriberi broke out in the second.

In 1911 Funk [18], of the Lister Institute, succeeded in obtaining a concentrate from rice polishings which was capable of curing polyneuritis in pigeons in doses of 20 mg. He put forward the theory that not only beriberi, but also scurvy, pellagra and possibly rickets were due to the absence of certain specific factors from the diet which he termed "vitamines." In the meantime Osborne and Mendel [10], in America, had shown that butter contained a growth-promoting factor essential for the development of rats (1910–13). This factor, now known as vitamin A, was discovered independently by ...

and cod-liver

milk sugar use

factor, which

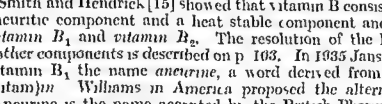
including milk, yeast, rice polishings, and wheat embryo. In 1915 McCollum and Davis [12] concluded that "There are necessary for normal nutrition during growth two classes of unknown accessory substances, one soluble in

fats and accompanying them in the process of isolation from certain foodstuffs and the other soluble in water, but not apparently in fats" The growth factor present in butter and cod-liver oil was called "fat-soluble A," owing to its solubility in fat solvents, and the other growth factor present in milk yeast, rice polishings and wheat embryo, was termed "water soluble B" It was soon realized that "water-soluble B" and the anti-beriberi or anti neuritic factor had similar properties and distribution, and it was therefore assumed that they were identical The two systems of nomenclature were therefore combined and the factor renamed *vitamine B*, and in 1920 the terminal *e* was omitted. Six years later Jansen and Donath [25] obtained 100 mg. of pure anti-neuritic factor from 3 kg of rice polishings At the same time Smith and Hendrick [15] showed that vitamin B consisted of a heat labile antineuritic component and a heat stable component and these were renamed *vitamin B_1* and *vitamin B_2*. The resolution of the latter into a number of other components is described on p 103. In 1935 Jansen suggested for pure vitamin B_1 the name *aneurine*, a word derived from *a*(nti poly) *neur*(itis) *vitam*)*in* Williams in America proposed the alternative name *thiamin* Aneurine is the name accepted by the British Pharmacopœia for vitamin B_1

In 1932 Windaus and his collaborators [32] isolated aneurine from yeast and determined the correct empirical formula of the vitamin Further work in 1934 by Windaus, Tschesche and Grewe [33] in Germany, and by Williams [40] and his school from 1934 to 1936, led to the elucidation of the chemical structure of the vitamin The final chapter in its history was written in 1936 by Williams and his co-workers [35], who brought their work to a brilliant conclusion by its synthesis. An alternative synthesis was published later by Todd and Bergel [717]

CHEMISTRY OF ANEURINE

Aneurine, or 2-methyl-5 (4-methyl-5-β hydroxyethyl thiazolium chloride) methyl-6 aminopyrimidine hydrochloride, has the following structure



Pyrimidine portion Thiazole portion

Aneurine is a colourless crystalline substance containing a molecule of water of crystallization and melting at 248°–250° C It is sensitive to ultra violet light of less than 290 mμ, with maximal photochemical decomposition at 255 mμ at pH7. The effect of heat is important on account of the possibility of the destruction of the vitamin in the ordinary processes of cooking and canning The rate and extent of destruction is markedly increased by the presence of wate ... stable at 100° C for twenty-four ... heat on aneurine depends not onl ... Thus at pH 5 4 for an hour one hundre ... borates, fifty-seven per cent in ... acetates, and three per cent in ... function of time temperature pH, and the presence of electrolytes Meat curing ingredients (sodium chloride, nitrate and nitrite, cane sugar and glucose) have no significant effect in accelerating destruction during heating [50] Copper catalyses the rate of destruction of aneurine, but iron, aluminium, zinc and tin do not [30] This is of some significance as these metals are present in cooking utensils

Destruction due to heating in the ordinary process of cooking is not very great provided the cooking temperature is not much above 100° C the reaction is not alkaline and the heating is not too prolonged Considerable inactivation occurs in pressure cookers The presence of other components in the foodstuff under consideration may facilitate the decomposition of the vitamin The destructive action of sulphites is of some importance since these are used in the preservation of fruit pulp and juices The sensitivity of aneurine to sulphites which inactivate it depends on the pH of the medium Thus decomposition is instantaneous at pH 6 at pH 5 about eight to twelve hours is required for complete decomposition at pH 3 there is relatively little destruction even after a period of months Aneurine is fairly resistant to heat in faintly acid or acid media Below pH 5 0 aqueous solutions are fairly stable to boiling [30] In sealed tubes at 100° 125° C when the pressure is considerable 0 1 per cent solutions at pH 3 6 are unaffected for

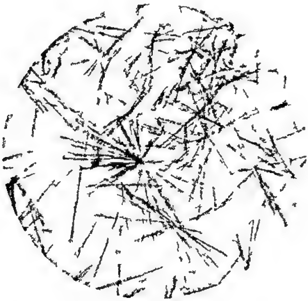
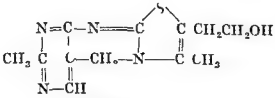


FIG 63 Crystals of Vitamin B Hydrochloride

short periods This is of importance in sterilizing solutions for parenteral injection Such solutions (pH about 3 5) may be heated for half an hour at 100° C or twenty minutes at 120° C with little loss of potency Aneurine is more stable in solution in the presence of five per cent glucose [551] Unless anti oxidants are used solutions of aneurine in ampoules suffer slow deterioration some sixty per cent decomposing after twelve months even when the amp

The
mil
to thiochrome [729 742]



Thiochrome

which shows an intense blue fluorescence under ultra-violet light, a reaction employed in the estimation of aneurine (p 241) *Thiochrome* is devoid of any antineuritic action

UNITS OF ANEURINE

In 1931 the Health Organization of the League of Nations [419] adopted an *international standard* for aneurine, consisting of an acid clay adsorbate of the vitamin from rice polish extract, prepared in a standard manner described by Jansen and Donath An International Unit was defined as the antineuritic activity of 10 mg of the adsorbate The International Unit is still retained in much of the literature, and until 1938 was generally considered to be equivalent to 2 micrograms of pure aneurine (*i e. 500 I U. = 1 mg*) In that year the International Conference on Vitamin Standardization recommended that the International Unit be defined as possessing the antineuritic activity *of 3 micrograms* of pure aneurine hydrochloride (i e 333 I U = 1 mg or 333,333 I U = 1 gram) The Permanent Commission on Biological Standardization of the Health Organization of the League of Nations adopted this recommendation The International Standard now consists of pure crystalline aneurine hydrochloride, which is kept at the National Institute for Medical Research Hampstead *The mean value of nine laboratories using* the rat growth method was 317 2 I U per milligram of aneurine hydrochloride with a molecule of water of crystallization This is the value, now approximated to 320 I U, accepted by the British Pharmacopœia

DISTRIBUTION OF ANEURINE IN FOODS

Aneurine is widely distributed in raw untreated foodstuffs, the richest sources being whole cereals, yeast, pork and pulses The cereals rank first as the most important source of aneurine in human diets On account of their cheapness and high calorie value they are consumed by most people Refined cereals and flours, however, suffer considerable loss in their aneurine content because the germ and bran are largely removed in the milling The greater part of the aneurine is concentrated in the scutellum the shield like tissue of the germ lying betwen the embryo and the endosperm [590] There is an increase in the aneurine content of cereals on germination, e g from 7 to 9 micrograms per gram in the case of wheat

White flour contains relatively little aneurine in comparison with wholemeal flour *It contains only 45 to 90 micrograms of aneurine per 100 grams*, i e about a fifth to a tenth of that originally present in the whole wheat
The production of white fl ... neurine,
little was lost in the now ... read"
(e g Hovis), a proprietary ... e flour
and one of wheat germ, is approximately equal to wholemeal bread in its aneurine content Stoneground flour contains the whole germ and endosperm and the inner layers of the pericarp, which give a cream coloured flour and not a brown one "Peeled wheat" is prepared by a flotation process which removes only the thin epidermis and leaves ninety eight per cent of the wheat berry It has a high content of aneurine and other members of the vitamin B complex [17]

In 1940 the Ministry of Food arranged for the fortification of white flour with aneurine to bring its content up to 0 083 mg per 100 grams of bread Owing to the lack of shipping space the Ministry forbade the milling of *white* flour after March 23rd, 1942, and the sale of white bread after April 6th, 1942,
and replaced white flour, w ... of the
wheat berry, by flour of eigh ... ch was
not fortified with aneurine, ... d con

tained an average of 300 to 400 micrograms of aneurine per 100 grams The loaf made from this contained 240 to 255 micrograms per 100 grams On October 1st 1944 a flour of 82 5 per cent extraction was introduced by the Ministry of Food In the early part of 1945 the extraction was lowered to eighty per cent to satisfy the public In the United States the millers may enrich white flour if they desire Such flour must be labelled enriched and satisfy the following specification Each pound must contain not less than 1 66 mg and not more than 2 5 mg of aneurine (or 0 47 to 0 7 g per 280 lb sack) not less than 1 2 mg and not more than 1 8 mg of riboflavine not less than 6 mg and not more than 9 mg of nicotinic acid or its amide and not less than 6 mg and not more than 24 mg of iron Vitamin D may be added between the limits of 250 and 1 000 U S P (International) units and calcium between 500 and 2 000 mg per pound of flour Enrichment was compulsory during the last war the order expired in October 1946 but since that date many States have made enrichment compulsory again

Legumes and nuts are important sources of aneurine Eggs contain a fair amount although the content depends on the diet of the bird Meat apart from pork is not a rich source although it may supply up to one third of the daily needs when it forms 10 per cent of the diet [194] Liver extracts may contain considerable aneurine

Yeast is an exceptionally potent source of aneurine and is frequently used as a dietary supplement when large quantities of the vitamin are required Marmite is a commercial yeast extract Brewer s yeast is more active than baker s yeast

Milk is a poor source of aneurine an average sample of raw cows milk containing about 40 to 50 micrograms per 100 c c Pasteurization of milk results in a loss of ten to twenty per cent of the aneurine content and commercial sterilization destroys some twenty six to forty five per cent [59] If the milk is concentrated by evaporation or drying the loss is greater still e g thirty to fifty per cent although in the presence of sugar as in sweetened condensed milk the loss is only ten per cent [63] Human milk is also poor in aneurine the content of which gradually increases until the twelfth week and then remains constant [759] At beginning of lactation human milk contains $3\ 4 \pm 1\ 45$ micrograms of aneurine per 100 ml by the second week it rises to $10\ 5 \pm 3\ 46$ micrograms in the third week and subsequently it rises to $13\ 8 \pm 4\ 47$ to $17\ 9 \pm 3\ 13$ micrograms [674] It can be increased to 24 to 30 micrograms by giving supplements of aneurine On a daily intake of 1 5 mg of aneurine the daily excretion in the milk is approximately

]

Freezing and Drying on Aneurine Many of the raw foodstuff but by the time the latter is prepared for the table it may have suffered some loss in its aneurine potency The chemical principles involved in the destruction of the vitamin

little inactivation in acid or neutral solutions heated to 100 C or just over for one hour Higher temperatures and increased alkalinity (increased *p*H) hasten the rate of destruction thus fifteen per cent of the aneurine in yeast is destroyed when it is heated for two hours at 130° C

The destruction of aneurine when foods are cooked in the ordinary way is not appreciable In the case of peas moderate amounts of soda do not seem to cause excessive destruction of aneurine [64] The average loss in the boiling of vegetables is about twenty to thirty per cent [205] This is reduced to ten per cent by cooking with as little water as possible and retaining much of the steam Much aneurine is dissolved out in the cooking water (twenty to thirty five per cent) and may be lost unless incorporated in soups and stews Root vegetables presenting less surface suffer less Potatoes

suffer less loss if cooked in their skins, retaining about ninety per cent of their aneurine. A considerable quantity resists aqueous extraction in starchy vegetables owing to adsorption on the starch grains. The loss is high if potatoes are steamed and the cooking liquid rejected, as a greater part of the aneurine is washed out [27]. The more succulent vegetables ("greens") do not retain so much of their aneurine on boiling. The widespread practice of using baking soda in cooking is to be condemned since it destroys much of the aneurine. In cooking rice all the water should be evaporated. Running water over the cooked rice to separate the grains washes out much aneurine. As much as sixty per cent of the vitamin may be lost in this way [26]. Pressure cooking, although it may be rapid, causes considerable destruction of aneurine if prolonged.

In the baking of bread the loss of aneurine is variously reported from twelve to thirty-five per cent [65, 66]; it increases with the time of baking, e.g. seventeen per cent at twenty minutes and thirty-three per cent at forty minutes. An average figure under normal conditions of large scale baking is twenty per cent. Dawson and Martin [65] give the following figures for the percentage losses of aneurine in the baking of various breads: white bread, eight to twenty-two; wholemeal ("brown bread"), thirty-five; National loaf, twenty-seven; germ bread, nineteen. A loss of up to thirty per cent can occur when baking soda is used [646], although normally little destruction is stated to occur [661]. When bread is toasted considerable destruction occurs in the outer part of the bread, although the total loss is not great. Toasting wholemeal bread for seventy seconds causes a drop in the aneurine content from 3.36 micrograms per gram to 2.29 micrograms per gram [49]. The cooking of oatmeal results in very little loss—five to ten per cent in half an hour [19].

The loss of aneurine that occurs in foods cooked in restaurants and cafeterias has been studied by a number of investigators, particularly in America. Losses varying from sixteen to seventy-five per cent have been reported [48]. Nagel and Harris [48] compute that twenty per cent is lost in the cooking water and twenty-five per cent between the time of cooking and serving. In the cooking of meat it has been estimated that there is from thirty to nearly sixty per cent loss on roasting and boiling and fifty per cent on braising [202], drying being a quick process results in less destruction, e.g. ten to forty-five per cent [194]. The loss in pressure cooking is approximately the same as in roasting [615], although if prolonged it may be considerable [29].

In the process of canning there should be little loss of aneurine due to the processing *per se* [67]. Any loss in potency usually occurs during the preparation of the food before it is put into the cans. In the blanching of fruit before canning thirty-six to ninety-nine per cent of the aneurine is retained [553, 557]. As a rule fruits are processed at 100° C., and meat and milk at 117°–120° C. or a little more, vegetables at 112°–115° C. Provided the medium is not alkaline there should be no appreciable destruction of the aneurine if the heating is limited to half an hour. It is understood of course that the juices as well as the solid food are consumed, as much of the vitamin (thirty per cent) is in the former. Arnold and Elvehjem [202] report a loss of up to twenty per cent in tinned meat, Rice and Robinson [906] thirty per cent in canned ham, and Mayfield and Hedrick [1] approximately seventy per cent in tinned beef. According to the latter workers losses are greater (eighty-five per cent) in glass jars. In the canning of vegetables losses of thirty-four to seventy-four per cent of aneurine may occur [898]. The loss in canning is increased on storage and may be as much as forty-five per cent after a year [23], although if the pH is suitably adjusted (e.g. 4.5 or less) there is said to be no loss of aneurine in the storage of canned fruits at room temperature [554]. In the preparation of corned beef losses of fifty to eighty per cent may occur [1].

Foods preserved by freezing suffer little or no loss in their aneurine content [67 69 70] The losses that do occur are due to the preliminary blanching or cooking rather than to refrigeration Thus in the processing losses of five to twenty five per cent may occur [22] It should be remembered that in the thawing process cell membranes are ruptured and that when frozen foods are cooked aneurine and the other water soluble vitamins are more readily extracted by the cooking water than from the fresh product

Dehydrated vegetables and meat were introduced as a wartime measure to save space in transport The loss of aneurine in dehydration is never more than about fifteen per … s during the blanching and not fron … neat the loss is from twenty five to … as high as fifty per cent are quoted [942]

In the preservation of vegetables by salting and brine preservation appreciable amounts of aneurine are retained [913] Considerable loss may occur in foods sulphited at a high pH As the legal maximum of sulphite is less than 4 000 p p m and this would be diluted with other foods on ingestion there is not much danger of sulphited food causing aneurine deficiency [659]

Aneurine Content of Foods The aneurine content of some of the more important foodstuffs in the raw and cooked states is given in the following table

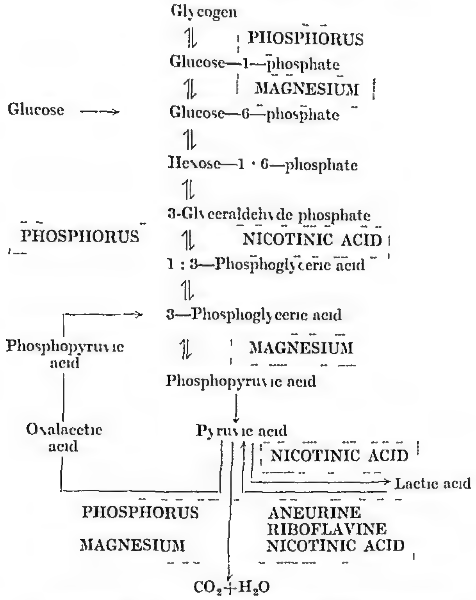
ANEURINE CONTENT OF FOODS

Foodstuff	Description	Micrograms of Aneurine per 100 g and or 3½ oz
VEGETABLE PRODUCTS		
Breads		
Maize		240
Rye	Whole grain	240 500
	Germ	225
	Bread	160
Wheaten bread	Whole grain (wholemeal)	225–450
	White bread (73% extraction)	45–90
	baking powder	31 63
	with malt	81 105
	With germ brown bread	240 270
	Without germ	195 240
	Bran bread	150
	Germ bread	240 510
	Milk bread	90
	Ministry of Food loaf (85% extraction (1942–44))	240 [illegible]
	Enriched (U S A)	240–400
	Doughnuts	280
Cereals and Cereal Products		
Barley	Whole grain	500
	Germ	4 200
	Pearled	1[illegible]0
Buckwheat		[illegible]10
Corn sweet		150
Maize	Whole grain	135 180
	Germ	1 380
Oatmeal	Whole grain	540–810
	Breakfast	420–810
Rice	Whole	60 290
	Polished	50
	Bran	1 [illegible]80–2 280
Rye	Whole	470–500
	Germ	2 230
Sorghum (Kaffir corn)	Black	270
	White	240
	Seed husk	735

Foodstuff	Description	Micrograms of Aneurine per 100 grams or 3½ oz
Cereals and Cereal Products—continued		
Wheat	Whole grain	510 1 080
	White flour	60 90
	Peeled wheat flour	580
	Germ " flour	360–390
	Bran	1 140
	Germ (commercial)	1 800 3 750
	Middlings	1 350–1 675
	Stone ground	270 330
	" " "white"	270 330
	85% extraction (National Wheat meal 1942–44)	300 400
	80% extraction	231 246
	"Enriched" (U S A)	440 500
	Self raising	20
Prepared Proprietary Cereal Foods		
Allbran	—	370 520
Bemax	—	2 625
Cerevim	Vitamin concentrate added	2 100
Corn flakes	Kellogg's	390–450
	(Vitamin concentrate added)	
	Post's	280 400
	(Vitamin concentrate added)	
Cream of rice	—	
Cream of wheat	Vitamin concentrate added	160
Force	—	410–680
Grape nuts	Post's	40
	(Vitamin concentrate added)	810
Oats	Quaker	580 700
Rice Krispies	Kellogg's	450
Shredded wheat	Kellogg's	190
Soya wheat	—	71
Fruits		
Apple	Raw	30 1
Apricot	—	30–4
Avocado	—	90 1
Banana	—	50 1
Blackberry	—	30–45
Black currant	—	30
Cherry	—	50
Gooseberry	—	150
Date	—	75
Fig	Fresh	60 75
	Dried	45–60
Grape	—	50
Grapefruit	Juice	50 75
Guava	—	45 150
Lemon	—	40–60
Lime juice	—	30
Loganberry	—	33
Melon	—	30 60
Orange	Juice	70 92
	Marmalade	20
Peach	Fresh	40
	Canned	70% retention
Pear	—	20–45
Pineapple	—	80 90
Plum	—	50 150
Prune	Dried	100 180
Raisin	Dried	150
Raspberry	—	20 30
Redcurrant	—	45
Strawberry	—	30
Tangerine	—	70 120
Nuts		
Almond	—	235
Brazil	—	500 1 180
Cashew	—	150
Chestnut	—	240–270
Cob	—	228–660

Foodstuff	Description	Micrograms of Aneurin per 100 grams or 3½ oz
Nuts—continued		
Coconut	Dried	trace
	Fresh	30–60
Hazel	—	400–600
Hickory	—	600
Peanut		694–1,050
	Roasted	300–400
	Butter	200–300
Pecan	—	500
Walnut	—	330–480
Vegetables		
Artichoke	—	75–180
Asparagus	—	180
	Canned	67% retention
Bean, baked	—	120
butter	Dried	480
haricot	—	156–400
	Cooked	120–180
green	—	80
kidney	—	210
string	—	198–450
runner	—	75–225
Beetroot	Boiled	10–30
Broccoli	—	130
Cabbage	—	30
	Dehydrated	410–590 (42–76% retained after preparation)
Carrot	Raw	60–70
	Canned	33
Cauliflower	Raw	100–150
	Cooked	90
Celery	—	30
Chives	—	120
Cress	—	90–150
Cucumber	—	30–40
Eggplant	—	45–70
Endive	—	50–75
Garlic	—	150
Kale	—	150
Kohlrabi	—	50
Leek	—	80–150
Lentil	—	120–630
Lettuce	—	60–75
Mango	—	60–90
Marrow	—	30–60
Mushroom	—	60–120
Okra	—	120
Onion	Stewed	30
Parsley	—	80
Parsnip	—	80–120
Pea	Fresh	400–800
	,, cooked	200
	Dry	550–590
	,, cooked	45–135
	Canned	40–70% retained
Peppers, green	—	30–70
,, red	—	30–70
Potato	Raw	90–180
	Dehydrated	250
	Chips	350
	Peeled and boiled	90
Pumpkin	—	50
Radish	—	40–60
Rhubarb	—	10–25
Soya bean	—	470
	Dried	1,140–1,200
Spinach	Raw	50–120

compounds until pyruvic acid is formed The successive stages in its oxidation (glycolysis) are probably as follows



The various steps in the breakdown of glucose are catalysed by enzymes which are activated by coenzymes. The enzymes are synthesized in the body, but the coenzymes, or at any rate their precursors, can only be made from dietary sources Among the coenzymes essential for the degradation of glucose are —

1 Adenosine triphosphate, derived from adenylic acid, which is a phosphate donor and acceptor

2 Diphosphopyridine nucleotide (codehydrogenase I) and triphospho pyridine nucleotide (codehydrogenase II), of which nicotinic acid is the precursor These coenzymes are hydrogen transporters

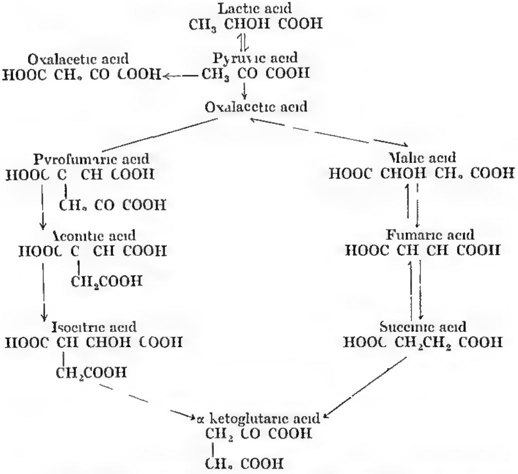
3 Magnesium

The oxidation and decarboxylation of pyruvic acid requires the enzyme coenzyme system carboxylase (protein aneurine pyrophosphate-magnesium) and cocarboxylase, or aneurine pyrophosphate There are other factors essential for the oxidation of pyruvic acid, including flavoprotein (p 294), codehydrogenases I and II, adenosine triphosphate and the cytochrome system

Pyruvic acid is not oxidized directly to lactic acid, but is probably first carboxylated to oxalacetic acid, which in turn is utilized in two cycles, the

citric acid and succinic acid [81]. The enzyme catalysing this reaction is carboxylase. The rate of pyruvic acid oxidation is controlled by the presence of aneurine. *In vitro* the synthesis of citric acid from pyruvic acid in the presence of kidney tissue is accelerated by aneurine, and *in vivo* aneurine deficiency in rats results in a decreased urinary excretion of citric acid [14, 89].

THE TRICARBOXYLIC ACID CYCLE



This is known as the tricarboxylic acid cycle. Some of these reactions have been shown to occur in bacterial metabolism and in isolated animal tissues such as brain, liver and kidney, but the exact pathways of carbohydrate metabolism in man have not been fully elucidated.

Aneurine becomes phosphorylated to cocarboxylase when added to many animal tissues. According to Goodhart and Sinclair [94] the white blood cells originating in the bone marrow convert aneurine into cocarboxylase, which is then probably combined with a protein. The circulating form of aneurine is the free substance or its monophosphate, not cocarboxylase, which is probably formed mainly in the liver. At any rate there is a massive synthesis of it in *the liver after the injection of aneurine. Cocarboxylase in the liver is hydro*lysed when necessary to replenish the blood aneurine. The kidney also phosphorylates the vitamin, the process being probably essential for its reabsorption in the kidney tubules. It is possible that insulin plays a part in the phosphorylation of aneurine: injection of insulin is followed by an increase in cocarboxylase and a fall in blood inorganic phosphate [561].

Since the liver and kidney possess the power of phosphorylating aneurine to cocarboxylase, it would be expected that some disturbance of phosphoryl

ation might occur in hepatic and renal disease This has been demonstrated by Williams and Bissell [914] They found that the injection of 15 mg of aneurine intravenously caused a *rapid increase* in cocarboxylase and free aneurine in the blood of normal subjects The aneurine level rapidly returned to normal, but the cocarboxylase *remained elevated* for an hour or so In patients with advanced hepatic cirrhosis there was an *immediate rise in the* free aneurine in the blood, but the increase in cocarboxylase was considerably less than in normal subjects *In cases of nephritis the* changes were intermediate between those in the normal subjects and those with hepatic disease Davis and Bauer [936] have also shown that there is some degree of aneurine *deficiency* in patients with hepatic disease, as shown by elevated blood pyruvic acid levels (p 242) They did not, however, find a raised blood pyruvic acid in patients with renal disease

Through its effect on the oxidation of pyruvic acid cocarboxylase may influence the various phases of carbohydrate metabolism since the oxidation of pyruvic acid causes the storage of a considerable amount of energy as adenosine triphosphate It may be indirectly concerned in the synthesis of glycogen from glucose and the conversion of fructose to glucose, reactions in which phosphorylation of the sugar are essential Cocarboxylase would also appear to be essential for the synthesis of carbohydrate from lactic and pyruvic acids It has been shown that the synthesis of carbohydrate from pyruvic acid is decreased in kidney slices of aneurine deficient rats and restored to normal by the addition of aneurine

Many investigations have been carried out to see if increased quantities of pyruvic acid and other intermediate products of carbohydrate metabolism can be detected in the blood of animals and human beings suffering from aneurine deficiency The presence of pyruvic acid in the blood can be demonstrated by an increase in the bisulphite binding power (B B S) of the blood Pyruvic acid contains a ketonic group (CO) and therefore combines with sodium bisulphite The test, not being specific for pyruvic acid, is given by other substances containing an aldehyde or ketone group, e g methyl glyoxal which is also an intermediate product of carbohydrate metabolism Pyruvic acid is best estimated not with sodium bisulphite but by means of its reaction with 2 4 dinitrophenylhydrazine [2, 4] It has been found in abnormal amounts in the blood of animals suffering from aneurine deficiency and the level returns to normal after treatment of the latter [83] This has also been demonstrated in human beings suffering from beriberi and related deficiency diseases [84, 85] Light muscular exercise in patients suffering from aneurine deficiency causes the level of blood pyruvate to rise still further [86] The blood pyruvate rises after exercise in untrained normal persons, but the blood lactate does not [102], in contrast to patients with beriberi and suffering from aneurine deficiency, who show both a raised blood lactate and pyruvate level Blood lactate and pyruvate are increased after severe exercise and at high altitudes [82] and in toxic, infective and hæmolytic states in infants [13] Chesler, Homburger and Himwich [919] noted a high post absorptive blood sugar, a rise in the blood lactic and pyruvic acids, and a lowering of the lactic acid pyruvic acid ratio in aneurine deficiency There is considerable difference of opinion as to whether the blood pyruvate level is of value for the biochemical detection of aneurine deficiency (p 242)

Statements on the effect of aneurine on the fasting blood sugar and on insulin hypoglycæmia are conflicting [98, 100, 101, 105, 106] In the normal human subject and in the diabetic it probably has no effect [555, 670, 754] Magyar and Resofski [16] found that aneurine had no effect on the arterio venous difference in blood sugar (which may be taken as a measure of the degree of utilization of glucose by the tissues) They state that aneurine facilitates the diffusion of insulin into the cells of the tissues In a human subject suffering from aneurine deficiency a rise in blood sugar and diminished glycogen storage in the liver is stated to occur [330], according to

Tonutti and Wallraff [106] glycogen is absent from the livers of aneurine deficient rats and is restored by injecting aneurine and glucose There is a decreased glucose tolerance in aneurine deficiency in man and animals [327, 330] Aneurine also influences the absorption of glucose from the intestine , the rate is decreased in aneurine deficiency [20, 730] The specific dynamic action of glucose is increased by aneurine , the specific dynamic action of a diet high in carbohydrate and containing adequate aneurine is twice as great as that of a similar diet lacking in aneurine [28]

The *most striking lesions in animals and human beings suffering from* a pure aneurine deficiency are in the nervous system, the cells of which utilize only carbohydrate for their energy [175] It is supposed that lack of aneurine results in inefficient metabolism of carbohydrate throughout the tissues including the nervous system The oxidation of carbohydrate by nerve tissue is not only depressed in aneurine deficient subjects [105], but also by alcohol, anæsthetics and narcotics, which are stated to increase the aneurine requirements of the organism considerably [678] It must not be supposed that aneurine functions specifically in nervous tissue only , it affects metabolism in general

Aneurine and Fat Metabolism In animals a diet poor in aneurine and rich in carbohydrate brings on the symptoms of aneurine deficiency more rapidly than a diet rich in fat or protein [141 142, 287] If the animals are offered a choice of diet they eat fat in preference to carbohydrate [225] When fat is substituted isocalorically for carbohydrate in the diet of rats suffering from aneurine deficiency, there is a decrease in bisulphite binding substances in the urine [581] The aneurine requirements of an animal are less on a diet rich in fat than on one containing much carbohydrate Yudkin [677] has shown that not only do fat and protein spare aneurine, but that in the complete absence of carbohydrate rats can dispense with aneurine altogether The rôle of aneurine in the metabolism of carbohydrate renders this aneurine sparing action of fat and protein intelligible since the vitamin is needed for the oxidation of carbohydrates, but not for that of fats or protein Increased consumption of fat does not cause a rise in the blood pyruvate [756]

According to McHenry [114] the presence of choline is necessary for the aneurine sparing action of fat Evans and his collaborators [112] have
aneurine sparing action, the efficiency of the
f the fatty acid chain, maximum protection
› containing eight carbon atoms McHenry
hat aneurine is essential for the synthesis of
fat from carbohydrate , other components of the vitamin B complex can augment the synthesis [478] and severally they determine the quality of the fat that laid down under the influence of aneurine containing less unsaturated

In man the results are equivocal Thus Widenbauer and Wieland [916]

any evidence for the aneurine sparing action of fat in man , in fact, the urinary excretion of aneurine was decreased in five out of six subjects when the fat intake was increased Their observations confirm the view that the amount of carbohydrate in the diet is an important factor in determining the daily requirements of aneurine These conflicting results are probably due to the difficulty of keeping human diets constant in one factor while varying another

The isocaloric replacement of carbohydrate by ethyl alcohol and an adequate diet in other respects is stated by Butler and Sarett [14] to result in an increased excretion of aneurine and they suggest that alcohol like fat and protein has an aneurine sparing action This has been confirmed in the rat [669] Alcohol like fat and protein requires less aneurine for its metabolism than carbohydrate Sulphadiazine also has an aneurine sparing action in the rat probably by interfering with a catalytic mechanism resulting from the inhibition of thyroxine synthesis [650]

Aneurine and Protein Metabolism An adequate supply of aneurine improves nitrogen retention in the rat [567] on an aneurine deficient diet the nitrogen balance becomes negative [576]

Aneurine and the Endocrine System **Thyroid** Most of the studies on the relationship between thyroid function and aneurine are experimental although a few clinical studies have been made The literature is well reviewed by Drill [279] and Blaizot [117] Experimental observations can be divided into (*a*) the effect of aneurine deficiency on the thyroid gland, (*b*) the effect of the administration of aneurine on animals given desiccated thyroid or thyroxine or the thyrotropic hormone of the anterior pituitary gland The literature on the effect of aneurine deficiency on the thyroid gland is conflicting Creatinuria occurs in animals deficient in aneurine but this does not result if the animal is first thyroidectomized [117] After prolonged aneurine deficiency structural changes in the thyroid gland have been described characterized by an increase in colloid [115] and by hyperplasia [230] followed by atrophy of the gland as the deficiency was prolonged Other investigators [240] state that no changes in the thyroid gland can be detected in animals suffering from aneurine deficiency and attribute the increased colloid formation to iodine deficiency Similarly it is variously stated that injections of aneurine stimulate the thyroid and also have no effect on the gland [116 432] Hyperthyroidism increases the requirements of aneurine (p 210) but the effect is not specific as hyperthyroidism results in increased metabolism which is known to increase the requirements of a number of vitamins including aneurine and ascorbic acid

The loss in weight and anorexia produced in animals by feeding thyroid gland or thyroxine can be corrected by the administration of aneurine [118 120 644] Doses of 100 micrograms of the latter can annul the effect of 0 2 mg of thyroxine in the experimental animal Experimental hyperthyroidism is accompanied by a fall in tissue cocarboxylase [120] Drill and Sherwood [118] showed that the effect of aneurine in preventing the loss in weight of hyperthyroid dogs is due to the increased caloric intake which it produces It also appears that not only aneurine but other members of the vitamin B complex are needed for the recovery of lost weight in hyperthyroid animals [278] Liver function including glycogen storage is also depressed in hyperthyroid dogs and this is intensified by removing the vitamin B complex from their diet A diet rich in the vitamin B complex delays but does not prevent the onset of damaged liver function [295] Hyperthyroidism decreases the amount of aneurine in various rat tissues particularly the liver Summarizing the animal work it may be said that in hyperthyroid animals there is an increased demand for aneurine which if not supplied results in a depletion of the body stores of the vitamin with resulting anorexia loss of weight decreased stores of glycogen and diminished hepatic function These changes can be prevented by aneurine and the B complex

A number of clinical observations on the subject have been made There is an excessive urinary excretion of aneurine in thyrotoxic patients [296] and Frazier and Ravdin [121] as well as Means [297] have pointed out that such patients often show symptoms suggestive of aneurine deficiency Williams and his co workers [296] observed that the blood cocarboxylase was below normal and the blood pyruvate and lactate elevated in thirty four

of forty patients with thyrotoxicosis The intravenous injection of glucose also resulted in a blood pyruvate level higher than normal Williams and Kendall [298] state that patients given thyroid tolerate it better if aneurine is given as well From tests on normal volunteers they concluded that the thyroid hormone is less effective in stimulating metabolism in a state of aneurine deficiency Two normal subjects were given approximately 0 5 gm of desiccated thyroid daily and placed on diets containing variable quantities of aneurine When the diet was adequate in the vitamin the B M R rose to + 25 per cent *it fell to between − 8 and + 11 per cent* when the vitamin was restricted and rose to + 25 to + 30 per cent when aneurine was again provided in adequate amounts

Aneurine and Acetylcholine Aneurine is an essential factor in the transmission of peripheral nerve impulses It augments the activity of

iesterase
606 39]
ylcholine
he action

of cholinesterase in animal sera but they point out that it is only effective in a concentration greater than that found in the tissues They also observed that the blood cholinesterase is increased in pigeons with aneurine deficiency Aneurine itself has no effect on smooth muscle but acetylaneurine the acetyl ester like acetylcholine causes it to contract [147] During nerve stimulation both aneurine and *acetylcholine are formed [885]* and *it has been* suggested that it is not the vitamin itself but the acetyl ester that is liberated Aneurine is formed in heart muscle on stimulating the vagus [647] If acetylaneurine like acetylcholine is a chemical intermediary in the propagation of the nerve impulse one would expect it to be rapidly removed from the site of action Acetylcholine is rapidly hydrolysed at the nerve ending by cholinesterase Actually the enzymatic hydrolysis of acetylaneurine by serum and brain extracts is very slow [124] In the presence of pyruvic acid and potassium ions aneurine effects the synthesis of acetylcholine in brain tissue [128] Most of the aneurine of nerve tissue is in the myelin sheath and this is the storage battery in which acetylcholine formation takes place In Wallerian degeneration of nerve there is a marked disappearance of aneurine [527]

By alkaline oxidation of nerve fibres aneurine can be demonstrated in *the myelin sheath* by *the fluorescence of the resulting thiochrome [923]* In degenerated nerve a diminution in the aneurine content of the myelin sheath can be demonstrated in twenty four hours The acetylcholine content of nerve tissue decreases in the aneurine deficient animal [924] Aneurine may play a part in the synthesis of acetylcholine [36 37 39] Von Muralt [39] has suggested the following scheme

adenosine triphosphate + aneurine = adenylic acid + cocarboxylase

This mechanism may be essential for the formation of acetylcholine The breakdown of adenosine triphosphate results in the phosphorylation of aneurine to cocarboxylase which catalyses the anaerobic and aerobic decarboxylation of pyruvic acid

Anaerobic

$$\underset{\text{pyruvic acid}}{2CH_3\ CO\ COOH}+H_2O\quad \underset{\text{acetic acid}}{CH_3\ COOH}+\underset{\text{lactic acid}}{CH_3\ CHOH\ COOH}+CO_2$$

Aerobic $\quad 2CH_3\ CO\ COOH+O_2-2CH_3\ COOH+CO_2$

These reactions provide the acetic acid for the acetylation of choline which is formed from the dephosphorylation of nerve phosphatides Adenosine triphosphate and cocarboxylase act as phosphate donor and acceptor and are associated with the breakdown of glucose acting as energy transmitters

Reproduction Aneurine plays some part in the reproductive mechanism of the rat since the fertility of this animal is seriously impaired if it is deprived

of the vitamin [757] Disturbances of lactation also result Mice kept on diets deficient in the v

Sure [189] reports
to rats produce partia
tion in the third genera
found that feeding ra ... er one generation
in interference with lactation loss of maternal instinct cannibalism and progressive loss of fertility These symptoms were prevented by giving 2 mg manganese chloride daily to the animals

Anœstrus is also produced in rats suffering from aneurine deficiency [757] *although it is claimed that this effect is due to concomitant inanition depress* ing the functions of the anterior pituitary gland [941] It has been reported that the male and female sex hormones and vitamin D delay the symptoms of aneurine deficiency [299] Large doses of aneurine are stated to depress the activity of the anterior pituitary gland as shown by diminished excretion of progesterone [300]

Aneurine and Phagocytic Function Studies in vitamin deficiencies have shown that adequate amounts of most vitamins are essential for normal resistance to infection Careful quantitative studies of variations in phago cytic power in different nutritional conditions have been made by Cottingham and Mills [939] They find that the phagocytic activity of the peritoneal fluid in mice is diminished by eighty per cent if the animals are suffering from a mild degree of aneurine deficiency When this is severe phagocytosis cannot be demonstrated Reduction in phagocytic power was also observed in mice suffering from a deficiency of other vitamins (pp 120 297 420)

Aneurine and Mineral Metabolism Perla and Sandberg [129 130] believe that there is a metabolic interdependence of the vitamin with man ganese, the latter acting as an oxidative catalyst in the utilization of aneurine in the tissues Perla [190] also observed that aneurine deficiency caused an *increased retention of manganese in rats and that the toxic manifestations* of an excess of aneurine in the diet could be prevented by small doses of manganese The results of studies on iron and copper were not sufficiently constant to be reported Manganese in minute amounts stimulates the carboxylase system (p 194) [763]

There appears to be some relationship between aneurine and zinc which like manganese can replace magnesium in the carboxylase complex [764] In beriberi the zinc content of the blood nails and skin falls to half the normal values [765] There would seem to be some correlation between the aneurine and zinc content of foodstuffs

Relationship to Other Vitamins The phosphorylation of aneurine is presumed to occur through the agency of adenylic acid (pp 193 199) and
aneurine but panto
rine deficiency there
e riboflavine content
of the tissues falling *considerably* mainly because of poor absorption [766] There is however an increase in the riboflavine content of the liver in animals deficient in aneurine Clinically it has been shown by Sydenstricker [767] that in cases of nutritional deficiency resulting from lack of the whole vitamin B complex the administration of massive doses of aneurine may precipitate symptoms of a deficiency of one of the other members of the vitamin B com plex e g nicotinic acid or riboflavine The administration of large doses of aneurine *e g 10 to 80 mg over a period increases* the urinary riboflavine excretion in man [768] and decreases the excretion of nicotinic acid [597] Large doses of aneurine given to rats precipitates a deficiency of pyridoxine [38] Vitamin A is stated to act antagonistically against aneurine since the symptoms of a deficiency of the latter are intensified by giving vitamin A In the rat however a deficiency of vitamin A results in an increase of blood pyruvic acid, which is a manifestation of aneurine deficiency and which

responds to the administration of aneurine [769] A possible physiological relationship exists between ascorbic acid and aneurine It is said that the onset of scurvy on a minimal intake of the former is delayed by small amounts of aneurine and that the antineuritic action of the latter is increased by ascorbic acid [671] The oral lesions in dogs deficient in aneurine have been relieved by giving ascorbic acid [778] The biosynthesis of the latter in the rat is influenced by aneurine [39] Aneurine deficiency is stated to be associated with a prolonged prothrombin time [595]

Absorption of Aneurine Aneurine is absorbed to a limited extent and mainly from the upper portion of the small gut It is doubtful whether it is normally absorbed from the large gut According to Schroeder and Liebich [132] it was not absorbed when administered through a cæcostomy opening and Alexander and Landwehr [951] could not detect its absorption when physiological amounts e g 2 mg were administered in an enema On the other hand Najjar and Holt [780] did observe absorption from the large gut but only when large quantities (50 mg) were given in an enema It may be re excreted into the gastric juice after absorption [52] The maximum quantity that can be completely absorbed when given by mouth is 2 to 5 mg [41 42 51] if quantities larger than this are given the excess is found largely in the stools or destroyed in the lower bowel In the aged absorption is apparently extremely limited throughout the gastro intestinal tract [781] Supplements of 1 mg taken three times a day are apparently completely absorbed as the aneurine content of the fæces is not increased The maximum amount completely absorbed and therefore the maximum economic intake of the vitamin by mouth is about 5 mg daily Doses of more than this which are often prescribed particularly in tonics are therefore wasted According to Friedemann and his co workers [42] the maximum quantity destroyed in the intestinal tract and tissues is about 4 mg daily in persons with normal intestinal motility the colon is the site of greatest destruction According to Alexander [58] 10 mg of aneurine is the maximal amount of the vitamin that the body can metabol ze daily It is apparently not absorbed per rectum [779]

Absorption appears to be largely confined to the upper gastro intestinal tract because it is influenced by food intake less being absorbed on an empty stomach than after a meal [51] This may be due to lower stability in the alkaline secretion of the duodenum *In vitro* tests show that aneurine is stable in gastric juice over a range of pH 1 5 to 8 0 but not in bile pancreatic juice and suspension of antacids [43] Rafsky and Newman state that the presence of hydrochloric acid in the gastric juice is not essential for its absorption [55] although it is also claimed that achlorhydria may impair it [44 237] Antacids such as magnesium trisil cate adsorb aneurine and prevent its absorption but kaolin does not [43 44] If live yeast is taken by mouth not only is very little aneurine absorbed from the yeast cell (about seventeen per cent) but the latter actually withdraws aneurine from the food in the gut [45 46] Yeast if given as a dietary supplement should therefore be heated first to kill the cells it is not sufficient to dry it

Absorption of aneurine may be diminished in gastro intestinal disturbances such as vomiting diarrhœa [183] ulcerative colitis and neoplastic disease Short circuiting operations of the intestine [135] internal and external fistulæ and strictures and in fact any pathological condition of the gastro intestinal tract may lead to diminished absorp
absorbed by patients with hepatic disease [72
the latter may be associated with dyspepsia ar
absorption [688]

Aneurine is probably not phosphorylated before absorption from the intestines [52] but is absorbed in the free state [640] Absorption probably occurs by simple diffusion because the amount absorbed is roughly propor tional to the intake [42] and because the initial rapid absorption is followed

by a much slower rate of absorption presumably after diffusion equilibrium between the lumen of the gut and the intestinal mucosa has been established [53]

The phosphorylation of aneurine occurs in all nucleated cells [94] those of the liver and kidneys being particularly active After aneurine is absorbed from the gut it reaches the bloodstream and is carried to the liver and kidneys where it is phosphorylated to cocarboxylase [763] The same organs can dephosphorylate cocarboxylase and supply the free vitamin to the blood [91] this is transported in the plasma to other tissues [94] which rephosphorylate it [763] or else it is excreted in the urine

Storage of Aneurine The body is unable to store aneurine for any length of time When the subject is placed on an aneurine deficient diet losses of aneurine from the tissues are not equal Depletion occurs most rapidly from the muscles and most slowly from the brain nervous system heart and liver The aneurine in skeletal muscle is intracellular and extracellular mostly the former The latter is freely diffusible and in equilibrium with that of the plasma [903] The concentration of intracellular aneurine is about 50 micrograms per 100 grams When the aneurine intake is increased a point of saturation is reached and the tissues do not store any further aneurine in spite of increased intake [140] The larger part is then stored in the muscles If more than the optimum needs of aneurine are ingested the excess is metabolized in the body or excreted in the urine Aneurine is present in the cerebrospinal fluid (range 0 01 to 6 5 micrograms) [409]

The total amount of aneurine in the body of a well nourished person is about 25 mg [785] The richest tissue is heart muscle (2 to 3 micrograms per gram) followed by brain kidney and liver (1 microgram per gram) and skeletal muscle (0 5 microgram per gram) [139 785]

The blood level range of aneurine is 4 to 10 micrograms per 100 ml whole blood [903] Using different methods of estimation a range of 2 to 17 micrograms has been reported [150 153 163 177 417 714 723 832] It is present in the red and white cells mainly as cocarboxylase (3 to 12 micrograms per 100 mg with an average of 7 micrograms) free aneurine is present mainly in the plasma (0 5 to 2 micrograms per 100 ml) [903] The blood aneurine varies widely at a given intake even in the same individual and is not related to the rate of [illegible] The concentration in the tissues is about twenty times that [illegible] platelets contain four to ten times as much [illegible] 922] but since only one per cent of the two former is found in blood their contribution to the total blood aneurine is only ten to twenty five per cent [520]

The aneurine level in the blood of the umbilical cord is nearly twice that of the maternal blood (11 6 micrograms per 100 ml in the former 6 79 in the latter) showing that the fœtus and newborn obtain their aneurine at the expense of the mother s reserves [340 506] In the fœtal tissues aneurine is present mainly as cocarboxylase [507]

Excretion The kidney concentrates aneurine from plasma perhaps twenty times or more [705] although there is no direct relationship between urinary and blood levels The fact that diuresis can affect aneurine excretion profoundly suggests it is a non threshold substance and renal clearance studies indicate that extensive tubular resorption of aneurine does not occur [903] About five to eight per cent is quickly eliminated unchanged in the urine and a greater part of the remainder is metabolized in the tissues [155] The bulk of excreted aneurine is free but a small amount is cocarboxylase [illegible] intake the excretion rises to nine to thirteen per cent [68 71 158] [illegible] hours [illegible] e [784] [illegible] linearly

Excretion continues for many days [illegible] related to the intake but is not a simple threshold phenomenon It varies from person to person and within very wide limits and is not determined

solely by the nutritional status of the individual [60] On normal diets the daily urinary excretion varies from 36 to 625 micrograms. It normally exceeds 100 micrograms daily and is usually around 200 micrograms or more [71]. The fasting rate of excretion during two hours before breakfast is [illegible] take of 600 micrograms [illegible] micrograms daily [71] [illegible] of excretion after four hours at 15 micrograms; this corresponds to a minimal excretion of 4 micrograms per hour given by other workers [73] Allibone and Finch [74] give the daily range of excretion of aneurine in children on a daily intake of 1 mg. as 10 to 400 micrograms in twenty-four hours. In the newborn excretion, varying from 4 to 22 micrograms daily, exceeds the intake for the first few days of life [75–77] In spite of considerable variation in the urinary excretion from person to person, this is highly characteristic of the individual. The amount excreted is independent of the urinary volume [61]. Even when equilibrium has been established at a highly constant aneurine intake there are considerable day to day variations One normal person may excrete twice or three times as much aneurine as another normal person on exactly the same diet [68] In any one day these differences may be considerably larger than the mean differences. When the aneurine intake is increased from 1 to 2 mg a day it requires a period of about six weeks for the excretion to reach equilibrium with the new intake level, half of this change occurs in the first ten days [68] The excretion of pyramin, the pyrimidine-like component of the aneurine molecule, is far more constant than the aneurine and varies from 130 to 250 micrograms daily on intakes of from 0 6 to 2 mg. of aneurine daily [68]. The relationship between pyramin excretion and aneurine intake is linear on normal intakes of 1 to 2 mg daily.

Since the major portion of aneurine administered in daily doses of more than 10 mg is rapidly excreted in the urine, since doses of more than 35 mg dc [illegible] e tissues, there seems to be no [illegible] ine parenterally [80]. After cor [illegible] the body discharges excess of the vitamin at a rate which can be represented by a linear equation [68, 80]

The intake and urinary output of aneurine as reported by various investigators are given on p. 204 :

There is no correlation between the excretion of aneurine and the number of non fat calories in the diet [580]

On a constant intake with physical activity constant the rate of aneurine excretion is not related to body weight, basal metabolic rate or surface area. It is generally lower at full activity (3,400 calories) than at limited activity or at rest [177]

The urinary excretion of aneurine is diminished in diabetes [80], during infections [786], in the aged [781], during exercise [911], and as a result of injury and hæmorrhage [88] Diminished excretion has also been recorded in patients suffering from alcoholism, disseminated sclerosis and sprue [82]. It is increased in thyrotoxicosis [296] and during the administration of mercurial diuretics [914], sulphonamides [108] and salicylates [893], although there is diminished excretion if salicylates are given over a prolonged period. The excretion in pregnancy is stated to be within the same range as that of normal women [103, 171], that is approximately 100 to 200 micrograms daily.

According to some workers aneurine is excreted in the sweat in a concentration of 9 to 15 micrograms per 100 ml. [787, 788] The loss is negligible under normal circumstances, but may become significant in those working in hot and humid environments and doing heavy work. Mickelsen and Keys [789] state that aneurine is present in negligible amounts in the sweat, e g. 0 2 micrograms per 100 ml.

Aneurine is present in the fæces, which contain on an average 78 micro-

Author	Intake of Aneurine		Urinary Excretion in micrograms in twenty four hours
	Micrograms	Micrograms per 1 000 calories	
Daum *et al* [98]	140	56	17
	200	80	16
	625	250	31
Elsom *et al* [131 713]	1 000	400	51
	574	280	49
Gifft and Hauck [71]	651	350	56
Hathaway and Strom [96]	1 500	600	100–224
	1 000	370–450	113
	1 000	370–450	116
Keys *et al* [92]	1 000	370–450	147
	780	180	58
	630	190	176
Keys *et al* [93]	700	210	21
	1 000	330	106
Mason and Williams [794]	1 000	330	92
Melnick and Field [105]	800	400	119
	860	—	175
Oldham *et al* [95]	640	360	65
	974	540	107
Papageorge and Lewis [73]	normal diet	—	309
Roderick *et al* [97]	1 180	400	331
	1 200	400	376
Sastri *et al* [580]	800 1 300	—	66–1 200 Av 339

grams per 100 grams although it varies considerably from person to person Friedemann and co workers [177] found that it varied from 0 29 to 1 22 mg daily on a daily intake of 2 77 mg Most of it is derived from the organisms of the gut as its concentration in the stools is independent of the intake [104 107 951] On a diet restricted in aneurine the fecal excretion always exceeds the intake The bulk of this aneurine is not available to the host as it is fixed in the bodies of the bacteria and cannot be washed out The fecal aneurine of rats is not available to another animal on a low aneurine intake [56] Even when the urinary excretion is almost zero aneurine is still present in the fæces In man it is doubtful if the aneurine in the bacteria of the fæces is available because if bacterial growth is inhibited with phthalylsulphathiazole there is no evidence of a decreased excretion of aneurine and the B vitamins in the urine [127]

PHARMACOLOGY AND TOXICOLOGY

Aneurine is diuretic [185] possibly through a central rather than a renal effect [187] Large doses given intravenously produce vasodilatation with a fall of blood pressure bradycardia and respiratory arrhythmia and depression [110 186] Smaller doses increase the tonus of the isolated heart [721] and delay the onset of fatigue in the isolated perfused muscle [574] In a concentration of 1 in 100 000 aneurine augments the effect of histamine on the isolated intestine [109]

In the concentrations in which it is found in the body aneurine may play a part in the transmission of nerve impulses (p 199) But in very high concentrations e g 150 mg /kg it has a curare like action that is it prevents the contraction of muscle when the nerve to the latter is stimulated without decreasing the contraction on direct stimulation [111 131 648 920] In concentrations that do not curarize i e 15 to 30 mg /kg aneurine blocks sympathetic ganglia [125] In large doses aneurine is said to increase oxygen consumption [185] although it is claimed that it only does this in aneurine deficient animals after food [186]

Relatively enormous doses of aneurine e g 50 mg /kg are tolerated by most animals without toxic effects Large doses intravenously produce a shock like state in pigeons [932] In man side effects have been reported after the parenteral administration of doses of 10 to 100 mg Doses much in excess of 10 mg daily are unwarranted as they are not metabolized and are excreted unchanged in the urine [58] Among the side effects that have been reported are vomiting epigastric fullness severe cramps collapse and respiratory distress Symptoms resembling anaphylactic shock with eosinophilia and ... [772 777] Many of the reactions occurred after several ... test is not conclusive evidence of sensitivity to aneurine as the latter may give a weal and flare in normal and non sensitive subjects [776] Cases of intolerance are comparatively rare Sudden death however has been reported following the ...

HUMAN REQUIREMENTS OF ANEURINE

The human requirements of aneurine have been calculated from (*a*) animal data (*b*) aneurine content of human diets (*c*) production and relief of symptoms of aneurine deficiency in man by diets containing known quantities of aneurine (*d*) excretion studies of aneurine

Human Requirements based on Animal Data From animal studies Cowgill [198] in 1934 arrived at the following formula

Daily aneurine requirement in milligrams $=$ constant $\times$ weight $\times$ calorie requirements
$= 0\ 00142 \times$ weight in kilograms $\times$ calorie intake $\times 0\ 003$
$=$ weight $\times$ calorie intake $\times 4\ 26 \times 10^{-6}$

According to this formula a man weighing 70 kg and leading a moderately active life (needing say 3 000 calories) requires approximately 0 9 mg of aneurine daily This formula of Cowgill s is for the minimum requirement of the normal adult It has been criticized on the ground that the fundamental relationship is not between aneurine and calories but between aneurine and carbohydrate intake [142] Since fat and protein are aneurine *sparing* (p 197) the requirements of aneurine are decreased on a high intake of fat and protein and a low intake of carbohydrate Signs of aneurine deficiency (p 238) have also been reported in volunteers subsisting on diets containing the quantity of aneurine calculated from the Cowgill formula [786]

Aneurine Requirements calculated from Dietary Studies Calculations have been made of the aneurine content of normal diets from food tables [199 200 210 213] They vary from about 0 6 to 1 5 mg It is however difficult to arrive at an exact figure owing to the wastage on the plate and during cooking the considerable variability of the vitamin content of the same food and the variations in the time and manner of cooking With all these variables considerable differences may be found in the aneurine content of the diets of two people living in the same house and doing their own cooking On an average some thirty per cent of the aneurine in the average diet is destroyed by cooking in restaurant cooking it may be as much as seventy five per cent or even more Stiebeling and Phipard [210] made an extensive survey of the diets of American families in 1939 and concluded that most people consumed more than 0 72 mg of aneurine daily and that half the subjects examined received 1 5 mg or more daily Some in the low income groups in America are said to have a daily intake of as little as 0 5 mg daily [799] Lane Johnson and Williams [792] carefully analysed repre

tive samples of food for the aneurine content, and concluded that sixty six to seventy-five per cent of the American population consumed 0 8 mg of aneurine per 2,500 calories This was before the "enrichment" of flour with aneurine (p 187), which would bring this figure up to at least 1 3 mg An Australian survey reveals a daily intake of 0 84 to 0 88 mg of aneurine [212]

Elsom and Machella [727] determined the aneurine intake of a number of normal subjects who ate as much food of varied type as they liked No restriction was placed on quality, quantity or price and the food was not spoilt by over-cooking The average consumption was 1 125 mg of aneurine daily, with a range of from 1 to 2 15 mg This serves to show that there are considerable variations in intake and probably requirements from person to person. A survey of Eastern diets reveals that the daily intake of aneurine may be as low as 0 3 to 0 6 mg without beriberi supervening [207, 728]

Holt [140] states that on a uniform diet, carefully selected to include a given amount of aneurine, the requirements are lower than on a diet chosen naturally to satisfy appetite and taste On selected "artificial" diets the minimal aneurine requirement lies between 0 13 and 0 17 mg /1,000 calories, whereas on a natural diet it is between 0 17 and 0 23 mg./1,000 calories. The same author considers that a range of 0 24 to 0 44 mg /1,000 calories protects against deficiency symptoms He considers these values are valid for all age groups, manual workers and pregnant women. Young [148] from a dietary survey in Canada on 385 individuals concluded that the daily aneurine intake averaged only 0 2 mg /1,000 calories for adults and 0 22 mg for children On these very low intakes there were no deficiency symptoms

Requirements based on Aneurine Deficiency Studies. The aneurine requirements of man have been determined by noting the appearance or removal of the manifestations of aneurine deficiency in subjects on graded intakes of the vitamin These manifestations are described on pp 238–240

The aneurine requirements of man, based on these considerations, are given in the following table .—

Author	Daily Aneurine Intake on which Deficiency Symptoms were Observed	Minimum Daily Intake Considered *Desirable for Physical Fitness*
Daum, *et al*, 1949 [144]	No disturbance of sensory and psychomotor functions on 0 25–0 3 mg per 1,000 calories	0 25–0 3 mg per 1,000 calories
Elsom, 1942 [786]	0 28 mg per 1,000 calories	0 35 mg per 1,000 calories
Foltz, *et al*, 1944 [931]	0 33 0 38 mg per 1,000 calories	0 33–0 45 mg per 1,000 calories
Friedemann *et al*, 1949 [177]	0 25 mg per 1,000 calories	—
Hathaway and Strom, 1946 [159]	No deficiency symptoms on 0 37 0 45 mg per 1,000 calories	0 5–0 55 mg per 1,000 calories
Keys *et al*, 1942–44 [796 797, 930]	0 23 mg per 1,000 calories (*No symptoms at this level*)	0 23 mg per 1,000 calories
Melnick, 1942 [798] 1944 [145]	0 26 mg per 1,000 calories	0 35 mg per 1 000 calories (0 5 mg per 1,000 calories for safe margin)
Williams, *et al*, 1939–43 [149, 327, 715, 793, 794]	0 2 0 95 mg	0 45 mg per 1,000 calories (0 6 mg per 1 000 calories for safe margin)

These estimates vary from 0·5 mg to less than 0·23 mg aneurine per 1,000 calories in relatively short term experiments and from 0·26 to 0·35 mg per 1,000 calories for longer term assessments. This wide range is due to the fact that periods of observation varied from a few weeks up to nine months and evidence of aneurine deficiency varied from clinical signs of frank deficiency to the least detectable changes. Many months deprivation of aneurine may be necessary for the appearance of deficiency symptoms, hence the necessity for long term nutrition experiments. Keys and his co-workers [790] included simple strength tests, responses during brief exhausting work, prolonged severe work and psychomotor tests of speed and co-ordination. They also estimated the glucose tolerance and blood pyruvate, lactate, glucose and hæmoglobin at rest, during work and after recovery. Tuttle and his co-workers [160] studied the reaction time of volunteers on various intakes of aneurine; when this was low the reaction time was increased. It was found that subjects whose food requirement was approximately 2,500 calories daily maintained their normal reaction time when the diet contained 0·625 mg of aneurine. Daum and her co-workers [144] noted changes in reaction time, aneurine excretion, maximum work output and the oxygen uptake for a specified amount of work when subjects were maintained on graded aneurine intakes. The aneurine intake giving the optimum physiological responses was considered to represent the desirable intake.

Requirements based on Excretion Studies. Aneurine is not stored to any extent in the body. Since it is excreted in the urine measurement of urinary excretion on varying intakes has been used as a method for calculating the requirements of the vitamin. Although broadly speaking aneurine excretion is correlated with the intake in a well nourished person there are considerable individual differences and considerable day to day variations on the same intake. One normal subject may excrete several times as much aneurine as another normal subject on the same diet. Normal aneurine excretions have been observed in beriberi [218]. The response to a test dose of aneurine has also been used. If a test dose of, say, 1 to 5 mg of aneurine is administered the amount excreted will depend upon tissue reserves; if these are low much of the dose will be retained and the excretion will be low. The difficulty is in the interpretation of the results; a range of normal values has yet to be recognized. Some workers have correlated the excretion of aneurine and its excretion in response to a test dose with the presence or absence of deficiency symptoms. Unfortunately, much of the data obtained from excretion studies has not been submitted to statistical examination and has been obtained from short term experiments [68]. Mickelsen and his co-workers [68] have shown that when the aneurine intake is increased from 1 to 2 mg it requires a period of six weeks for aneurine excretion values to come to equilibrium with the new intake level. These workers claim that more reliable information is obtained from a study of the excretion of pyramin rather than aneurine (p. 244).

Holt [140] and Najjar and Holt [780] state that the 'point of minimum aneurine excretion' is closely related to the beginning of sub-clinical aneurine deficiency, and they have calculated the minimum aneurine requirements by gradually reducing the aneurine intake and noting when the excretion falls to a minimum value.

Alexander and Landwehr [58, 161] have calculated the human requirements of aneurine by finding the difference between intake and excretion. The difference is the amount of aneurine utilized in the body. The daily aneurine intake of a thirty-five year old man weighing 180 lb and consuming a 2,400 calorie diet was 1·3 mg and the excretion calculated as aneurine and pyramin-like was 0·24 mg. The balance 1·06 mg, or 0·44 mg aneurine per 1,000 calories was assumed to represent the daily aneurine requirement. When large supplements of aneurine were administered to the subject

Author	Type of Observation	Estimated Daily Aneurin Requirement
Alexander and Landwehr 1946 [58 161]	Difference between intake and excretion	0 44 mg per 1 000 calories
Daum *et al* 1948 1949 [99 144]	Changes in urinary excretion on graded intakes	0 25–0 3 *mg* per 1 000 calories
Holt 1942 [140]	Minimal aneurine intake for fasting level to fall to zero value	0 26–0 31 mg per 1 000 calories
Mason and Williams 1942 [715 794 795]	Excretion of 100 ± 10 micrograms in twenty four hours and recovery of 20 ± two per cent of test dose of 1 mg i m	0 4–0 45 mg per 1 000 calories
Melnick 1942 [782 798]	Intake noted at which aneurine excretion fell precipitously intake noted at which prompt response in urinary excretion occurs after test dose of 5 mg	0 35 mg per 1 000 calories (0 5 mg recommended)
Oldham *et al* 1946 [95]	Urinary excretion and response to test dose on graded intakes from 0 14 0 51 mg per 1 000 calories	1 mg or 20 micrograms per kg of body weight
Widenbauer and Wieland 1935 [208]	Graded dose of aneurine given to a subject not excreting the vitamin Daily intake noted when aneurine excreted in urine and blood level rose to 2 11 mg per 100 ml	0 37 0 55 mg
Williams Mason and Wilder 1943 [149]	Critical level associated with urinary excretion and biochemical deficits in carbohydrate metabolism and presence of deficiency symptoms	0 45 mg per 1 000 calories (0 6 recommended)

approximately the same figure 1 09 mg was obtained Alexander and Landwehr concluded that the aneurine present in the fæces is in the bodies of the intestinal organisms and not available to the host, fæcal aneurine is therefore of no significance in aneurine metabolism studies (p 204)

	Calories	Daily Aneurine Intake in mg N R C	Daily Aneurine Intake in mg B M A
Man (156 lb or 70 kg)			
Sedentary	2 400	1 2	1 0
Moderately active	3 000	1 5	1 2
Very active	4 500	1 8	1 7
Women (125 lb or 50 kg)			
Sedentary	2 000	1 0	0 8
Moderately active	2 400	1 2	1 0
Very active	3 000	1 5	1 2
Last half of pregnancy	2 400	1 5	1 1
During lactation	3 000	1 5	1 4

Human Requirements of the Adult Summarizing the results obtained by the various methods described it would appear that the minimum human requirements of aneurine are in the region of 1 mg daily This should be increased to allow for losses in cooking and wastage on the plate The actual requirement depends upon the number of non fat calories consumed and will therefore depend on occupation, metabolic rate, sex and other factors Even in the normal subject there are wide fluctuations in requirements The League of Nations Committee on Nutrition (1939) advised a minimum intake of 1 mg of aneurine daily [211] The Food and Nutrition Board of the National Research Council, U S A, 1948 [800] and the Nutrition Committee of the British Medical Association (1950) suggest the daily aneurine intake given in the table at the bottom of p 208

The Nutrition Committee of the British Medical Association (1950) adopted a basic estimate for all population groups, except nursing mothers, of 0 4 mg daily per 1,000 total calories and 0 6 mg daily per 1,000 non fat calories The intake suggested for the nursing mother is 1 4 mg daily

Requirements of Infants and Children The Committee on Food and Nutrition of the National Research Council, U S A (1948), suggest the following daily allowances of aneurine from infancy to adolescence [800]

	Calories	Daily Aneurine Intake in mg
Under 1 year	110/kg	0 4
1– 3 years	1,200	0 6
4– 6 ,,	1,600	0 8
7– 9 ,,	2,000	1 0
10–12 ,,	2,500	1 2
Girls 13–15 years	2,000	1 0
16–20 ,,	2,500	1 2
Boys 13–15 years	3,200	1 5
16–20 ,,	3,800	1 7

The Nutrition Committee of the British Medical Association (1950) suggest a daily intake of 0 4 mg aneurine up to 1 year of age, 0 6 mg between 2 and 6 years, and 0 8 mg between 7 and 10 years

The requirements of infants have been calculated from the aneurine content of mothers' milk [219 759 901], which varies from 0 6 micrograms per 100 ml in colostrum to 36 micrograms in milk, with an average of about 20 micrograms Calculations from these data vary from 0 1 to 0 6 mg of aneurine as the daily requirement with an average of 0 3 mg daily

If the infant were fed on cow's milk (average 0 035 mg aneurine per 100 ml) the average daily aneurine intake would be about 0 3 mg daily Thirty per cent or more may be destroyed on pasteurization As the metabolic rate of the infant and child per unit of body surface is greater than that of the adult, the aneurine requirements are relatively greater

The requirements of infants have also been calculated from excretion studies [162, 220, 711] Knott [711] increased the daily aneurine intakes of infants from 60 micrograms to 0 9 mg daily and noted a marked rise in the excretion when the intake reached 0 24 mg Holt and his co workers [162] calculated the requirements by means of a urinary excretion procedure which

intain urinary excretion at

which in the adult approxi

ficiency (p 238) On this

basis the requirement of infants varied from 0 14 to 0 2 mg daily

Ætiology It has been generally supposed that beriberi is a deficiency disease due to lack of aneurine Deficiency diseases, however, are never limited to lack of a single factor and, although there is an aneurine deficiency in beriberi, the disease is probably a multiple deficiency syndrome However a deficiency of aneurine is almost certainly a major cause, as Burgess [196] has shown that in prisoners of war beriberi was seldom seen when the aneurine/non fat calorie ratio was over 0 3 mg per 1,000 calories, when it fell below that figure the incidence of beriberi varied inversely with it It is not certain whether the neuropathy in beriberi is due directly to aneurine deficiency or to an intoxication with pyruvic acid and allied intermediate products of carbohydrate metabolism It is now recognized that the treatment of beriberi is more satisfactory with aneurine and foods or concentrates rich in the B complex than with pure aneurine alone Recent observations on induced aneurine deficiency (p 238) have shown that it is impossible to produce beriberi experimentally by diets poor in aneurine only Polished rice, which is the staple food where beriberi is endemic is not only deficient in aneurine but also in vitamins A, D, E and B_6 riboflavine, nicotinic acid pantothenic acid, choline, calcium and iron A deficiency of vitamin A and riboflavine in animals has been shown to produce degenerative changes in the spinal cord and peripheral nerves (pp 42, 295) Clinically few patients pre ... ngle avitaminosis, actually any ... several Certain manifestations ... ness nervous irritability, vague malaise, lassitude, mental confusion, depression and inability to concentrate

Cardiac and neurological lesions can be produced in monkeys on diets deficient in aneurine [178, 181] The condition however is not exactly analogous to that of human beriberi

Certain Indi ... as a cause of acute beriberi ... to be caused by ... taken from the breast Methylglyoxal, which has been found in the blood of patients with beriberi, is thought by some to be this toxin Stannus [762] who accepts the toxin theory, suggests that the methylglyoxal is formed during the breakdown of carbohydrate in skeletal or heart muscle and that in the absence of glutathione as a co enzyme it cannot be broken down by glyoxalase He postulates the possibility of a primary deficiency of glutathione in beriberi Haynes and Weiss [699] however, were unable to produce the cardiac manifestations of aneurine deficiency by the injection of methylglyoxal pyruvic acid or lactic acid

It is not known for how long the diet must be depleted of aneurine for the onset of beriberi, although it is stated that the symptoms appear after about three month ... [195, 709] Certain predisposing factors play a part ... These include poverty ... pregnancy ... digestive ... Dixon [734] points out that many Chinese women app... nancy but are often *in extremis* towards the end from beriberi The frequent association of malaria and beriberi commented upon by Cowgill [198] is due to the heightened metabolism of malaria increasing the vitamin requirements

Cases of beriberi resulting from inadequate vitamin absorption as a consequence of prolonged vomiting in such conditions as pyloric obstruction prolonged diarrhœa, fistulæ of the gastro intestinal tract or short circuiting of ... The di... can also result from unbalanced die... alco... 235] ... forms of beriberi have been described Eac... however, pre... l variations, and mixed forms are seen

Infantile Beriberi This is common in the East among infants in the first few months of life and is characterized by a very rapid onset and acuteness so that an apparently healthy child may die rapidly from the disease Cardiovascular symptoms predominate This has given rise to the assumption referred to on p 212 that it is due to toxic metabolites [810] Bray [811] gives a good clinical description of the condition The infant suffers from anorexia and is disinclined to feed from the breast milk is regurgitated but water is not it is restless and tender over the abdomen particularly over the liver , abdominal distension is present and is accompanied by colicky pain vomiting and paroxysmal screaming constipation diminished excretion of urine and water retention occur The latter leads to œdema (Fig 72) and an increase in weight so that the infant looks plump although wasting occurs later There follows tachycardia (200 per minute) tachypnœa dyspnœa and aphonia due to œdema of the larynx The latter is responsible for the peci
Lat
of t
tissues become filled with fluid to produce a generalized œdema Finally come signs of increased intracranial pressure with meningism rigidity twitchings drowsiness coma and death Each phase may only last a matter of hours and the whole condition a day or two The sudden paroxysms of pain cause the body to be held te[illegible]
not occur Infantile beriberi i
described on the Continent and sh
The mortality in the East before
ings (tikitiki) was very high (seventy four per cent) but this has been reduced considerably since the introduction of specific treatment Post mortem there is enlargement of the right ventricle and effusions into the pleural pericardial and abdominal cavities In addition there may be œdema or congestion of the liver spleen and kidneys and œdema of the brain and lungs Intercurrent disease often obscures the picture

Women with manifest signs of beriberi may nurse infants having no apparent signs of infantile beriberi and conversely seemingly healthy women
the disease [810] Such women
nably in the latent stage Con
the Orient although it has been
reported in America [944] The child is
manifestations aphonia tachycardia and cardiac enlargement Treatment of infantile beriberi consists of giving 10 to 20 mg of aneurine daily and improvement of the diet of the mother Fehily [810] states that the manifesta
Chinese in Hong Kong
child bearing
the following must be
considered overfeeding the results of which may resemble the vomiting of infantile beriberi bronchitis or bronchopneumonia which are often complications of infantile beriberi dyspepsia meningitis nephritis peritonitis which the gastro intestinal syndrome of infantile beriberi may mimic diphtheritic paralysis which may be suggested by the dysphagia aphonia and symptoms of circulatory failure in infantile beriberi laryngysmus stridulus which may be suggested by the cyanosis and dyspnœa of infantile beriberi although there is no crowing and Chvostek s sign is negative congenital syphilis which may be considered in view of the enlarged liver œdema and loss of weight Unless the diagnosis is entertained infantile beriberi may be easily overlooked in regions where the disease is not endemic

The *adult* or *chronic type of beriberi* in contrast to the infantile is usually insidious in onset except in cases of acute cardiac beriberi Clinically the disease is characterized by a triad of symptoms—cardiovascular disturbances neuritis and œdema—and various forms are termed dry (neuritic para

FATAL BERIBERI IN A CHILD

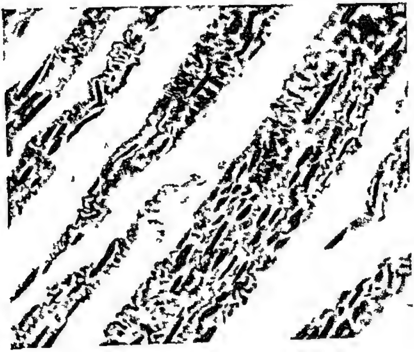


Fig. 64

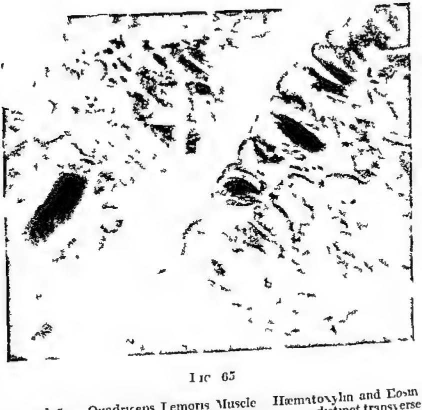


Fig. 65

Figs. 64 and 65. Quadriceps Femoris Muscle. Hæmatoxylin and Eosin × 85 and 380. Muscle fibres are atrophic. Some have an indistinct transverse striation and a wave-like spiral appearance similar to that seen in amyotonia congenita.

A FATAL CASE OF BERIBERI IN A CHILD

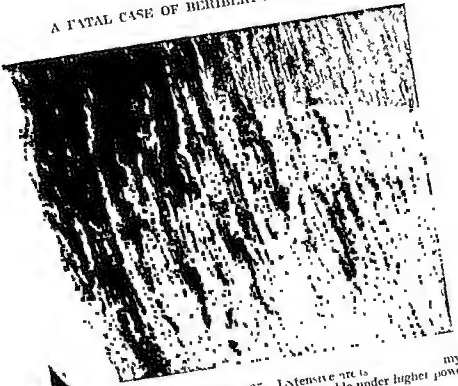


FIG 66 Spinal Cord Weigert Pal × 85 Extensive areas [illegible] myelinisation Swellings of the myelin sheaths are visible under higher power

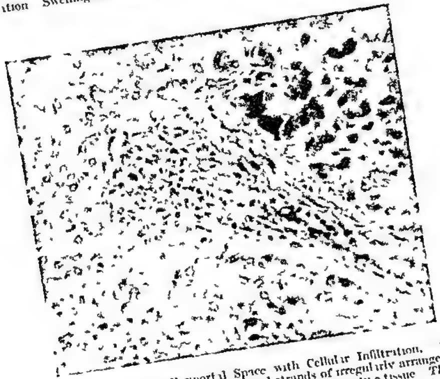


FIG 67 Liver showing Periportal Space with Cellular Infiltration. × 380 Under low power there are patches and strands of irregularly arranged liver cells separated by a network of broad strands of connective tissue The liver cells show some atrophy and vacuoles in the cytoplasm The periportal tissue is increased, consisting mainly of fibroblasts and collagen fibres infiltrated with small round cells.

plegic), "wet" or "cardiac," according to the prevailing symptoms. Mixed cases are also quite common. Neuritis is perhaps the most constant finding although cardiac symptoms may be present. Early complaints are a feeling of fatigue, cramps in the legs, heaviness and stiffness of the legs with areas of paræsthesia and tenderness along the nerve trunks. Soon after the patient may notice headache, insomnia, anorexia, dyspnœa, tachycardia, nervousness, irritability, depression, lack of interest and initiative, and tenderness of the

be poor and

Circulatory

unexplained

tachycardia, variable cardiac murmurs and a slight rise of blood pressure. These symptoms are vague, non specific and ill defined and seldom give rise to a diagnosis of beriberi.

If deficiency is prolonged the major manifestations of beriberi slowly appear. In *dry beriberi* (see Fig. 69) the nervous system is primarily affected.

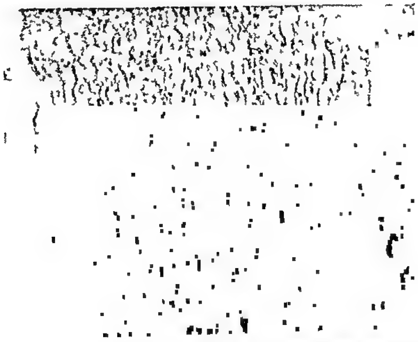


FIG. 68. A Fatal Case of Beriberi in a Child. Thickened endocardium of pulmonary conus. × 150. The subendocardial connective tissue is increased and contains many elastic fibres.

The clinical picture is one of ascending, symmetrical bilateral peripheral neuritis. Initially weakness, stiffness and cramps in the legs are complained of, walking for short distances is unimpaired, but weakness is apparent after prolonged exertion (e.g. a mile walk) when the patient's legs will suddenly collapse under him. Later distances of a hundred feet may be sufficient to cause collapse. There may be a burning sensation in the feet,* and a numbness round the dorsum of the foot and ankle with a weakness in dorsiflexion of the ankle joint. Vibration sense may be diminished. The achilles and patellar reflexes are increased at first, then

The ankle jerks are lost early, a sign of d

spreads upwards, first involving the extensor mu

... of the calf and finally the extensors and flexors of the thigh, which ... The affected muscles

on palpation

* ... sed elsewhere (p ...) ... it is probably not ... administered

Deep sensation elicited by compression of the tendo achilles (Abadie s sign)

Fig 69 Dry or Paraplegic Beriberi The arms and legs are chiefly affected The leg muscles have atrophied and the patient exhibits a characteristic posture

Fig 70 Beriberi Recovery from dry beriberi showing hyperextension of the legs with posterior displacement of the tibia due to laxity of the ligaments

are experienced followed by loss of tendon reflexes wrist drop (see Fig 71) hyperesthesia and anæsthesia Palmar and plantar erythema have been reported [203] The grip becomes so poor that the sufferer cannot button his clothes or pick up small objects and may find difficulty in feeding himself although there is rarely paresis of the muscles of the face tongue or pharynx There is loss of deep sensation The gait becomes ataxic since the patient loses the power to raise the toes and to avoid scraping them he walks by lifting the hips swings the legs which are held wide apart (Fig 69) and assumes a characteristic steppage gait That has been likened to walking in wet clothes or stiff clay The gait is also protective because of the tenderness of the feet The ataxia is due to muscular weakness and not inco ordination

As the disease progresses the patient becomes bedridden and suffers great pain from the pressure of the bed and clothes on tender muscles Severe

pains in the extremities, sufficient to prevent sleep, were experienced by prisoners of the 1939–45 war suffering from beriberi. Epicritic sensation is first affected, then temperature, pain and vibration sense. The muscles of the upper extremity, trunk and diaphragm may be involved, and muscular contractures and lack of muscular co-ordination may occur. Loss of sphincteric control does not occur until very late. The spinal cord may be involved with symptoms of spastic paraplegia, a positive Romberg test and a positive Babinski reflex [203]. The initial mild mental symptoms may be followed by mental confusion similar to that seen in toxic infectious states; insomnia, nervousness and emotional instability may also be present. Months may elapse before severe symptoms occur, which may be precipitated by an infection or severe privation. The eighth nerve may be affected, although rarely, with resulting tinnitus and deafness. Lesions of the optic nerve have

FIG. 71. Dry or Paraplegic Beriberi. Wasting of the extensors of the wrist and wrist drop.

been described [146, 193, 249, 751, 753] and were classed as prominent symptoms by Japanese writers earlier in the century. They include loss of visual acuity, bilateral central scotomata, concentric contraction of the field of vision, temporal pallor of the disc, some papilloedema, retrobulbar neuritis and optic nerve degeneration. These, however, may be due to a multiple vitamin B deficiency.

Cardiovascular and respiratory symptoms predominate in acute cardiac beriberi, which is described as "wet" if oedema is present. The first detailed and accurate description of the beriberi heart was made by Aalsmeer and Wenckebach [921]. The clinical picture is primarily that of cardiac over-activity and congestive heart failure and is similar to the clinical picture in thyrotoxicosis and arteriovenous aneurysm. The principal manifestations are dyspnoea and orthopnoea, palpitation on exertion, precordial pain, tachycardia and oedema [241, 244]. This fulminating acute type, known as *shoshin*, or "acute pernicious" beriberi heart [241], is a serious threat to life, many patients dying suddenly of heart failure if treatment is not instituted

at once The "beriberi heart" has long been known to clinicians, working in the East The heart is enlarged both to the right and left, although mainly to the right, the liver swollen, tender and pulsating the veins of the neck engorged, and the pulse small, rapid and thready The carotids pulsate violently and pulsation is visible or palpable in the epigastrium and jugulars Breathing may be so laboured as to suggest respiratory obstruction Numerous functional murmurs and signs of pulmonary congestion are common There may be a transient elevation of the systolic and diastolic blood pressures the pulse pressure and venous pressure are increased, and the electrocardiogram may show distinct changes (p 236) It is of low voltage and there is an indefinite inverted or flattened T wave in leads I, II and III, deviation of the RST segment, shortening of the P–R interval and prolongation of the Q–T interval These changes are non specific and quickly disappear on treatment On palpation a bounding quality is noted in the larger arteries and "pistol shot" sounds may be heard on auscultation Systolic murmurs and a loud sharp second sound over the pulmonary area may be heard The heart sounds have also been likened to the beats of a pendulum clock, i e, they are evenly spaced The pulse quickens rapidly on exertion and the pulse pressure is high because of a lowering of the diastolic pressure The skin is usually warm, moist and of normal colour, and cyanosis is rare Circulatory failure may be right or left sided Weiss and Wilkins [242] and Blankenhorn [215] examined a number of "beriberi hearts" in America and in contrast to Eastern beriberi, the heart was not always enlarged and rapid circulation was not constantly present

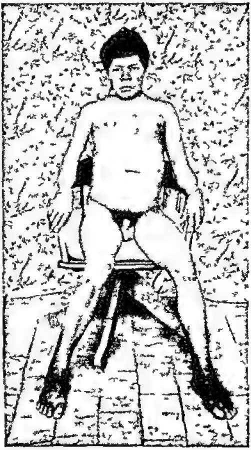


FIG 72 Wet or Œdematous Beriberi Note the ascites with obliteration of the umbilicus and œdema of the legs scrotum and face

Œdema is conspicuous in cases of "wet" beriberi (Fig 72) beginning in the feet and legs and extending up the body to the face, eventually leading to ascites, hydrothorax and hydropericardium This generalized œdema may mask the muscle wasting, and oliguria occurs while the œdema is developing There are apparently no renal changes accompanying beriberi œdema, which is firmer than that of nephritis and of a hydrostatic nature The rapid blood flow, warm extremities, flushed colour and increased pulse pressure indicate a generalized arteriolar dilatation The rapid blood flow returns the blood to the right side of the heart at an increased rate The heart being weaker than normal fails to deliver the blood to the lungs as fast as it is received, and congestion of the viscera commences If pulmonary œdema develops the right heart fails and its chambers dilate There is precordial distress or pain

some degeneration of the sheaths of scattered fibres in the anterior and posterior nerve roots and posterior columns may occur. Changes in the ganglion cells of the medulla and pons have been described [217].

The muscles supplied by affected nerves are considerably atrophied and histologically there is evidence of cloudy swelling, fatty degeneration, loss of cross striation and shrinkage of sarcoplasm.

The post mortem diagnosis of beriberi rests on (a) dilatation and hypertrophy of the heart, usually on the right, without evidence of organic cause; (b) visceral congestion; (c) œdema; (d) degenerative lesions in the peripheral nerves and spinal cord; and (e) absence of any other cause of death.

Prognosis and Treatment of Beriberi. The course is progressive unless treated and sudden death may occur from heart failure or secondary infection. The mortality varies from five to fifty per cent, according to the severity of the condition and the treatment used. Evidence of severe cardiac involvement, diaphragmatic paralysis and serous effusions are of serious import and demand instant treatment. Once remission has occurred the prognosis is good if the patient is placed on a diet rich in the B vitamins. After treatment and recovery there is remyelinization and regeneration; many of the nerve fibres are slowly restored to normal, although this is far slower than the clinical improvement.

For prophylaxis foods rich in aneurine and the vitamin B complex—cereals, peas, beans, peanuts and yeast—should be incorporated in the diet. It has been suggested that rice be enriched with aneurine, nicotinic acid and iron in areas where beriberi is endemic [209]; the provision of food yeast (*Torula utilis*, p. 193) has also been suggested. For many years an extract of rice polishings (*tikitiki*) has been used in the East as a preventive. The diets of patients in endemic areas suffering from debilitating diseases, chronic alcoholism, pellagra, sprue, cirrhosis, tuberculosis and other infections should receive special consideration.

The treatment of beriberi will depend on the severity of the condition. The acute state is a medical emergency, treated with bed rest and parenteral injections of 10 to 20 mg. aneurine daily; doses in excess of this are wasteful and unnecessary. As recovery sets in the dose can be reduced to 5 to 10 mg. given by mouth, unless impaired absorption is suspected. Venesection may be necessary to relieve the right side of the heart, and serous effusions may need paracentesis. In the chronic case 5 to 10 mg. of aneurine daily is adequate. At the same time the diet, which should be a high protein, high calorie one (4,500 calories), should be supplemented by foods rich in aneurine and by yeast (heat treated) or yeast extracts, or liver or rice polishings.

Specific treatment for the limbs may be needed. The patient should be rested in bed and the bedclothes should be kept off the limbs with a cradle; gentle passive movements of all joints and paralysed limbs should be performed several times a day, and active movements encouraged as soon as possible. Massage and faradism can be employed after tenderness has gone. Analgesia may be required for pain and night cramps treated with quinine 3 to 6 grains night and morning.

In the cases of infantile beriberi 5 to 10 mg. of aneurine is given parenterally [illegible] fant is breast fed [illegible] nt is dramatic; improvement [illegible] pain is relieved in a few days, but complete clinical recovery may take [illegible] weeks or months. Sometimes residual neurological changes remain in cases of complete degeneration of the ganglion cells and axis cylinders.

OTHER MANIFESTATIONS OF ANEURINE DEFICIENCY

Factors conditioning Vitamin Deficiency. A state of vitamin deficiency, either mild or gross, may develop from a deficient intake due to a faulty diet

in which case the deficiency may be said to be primary or it may result from factors other than an inadequate diet that is from conditioned deficiency A conditioned deficiency is caused by factors interfering with the ingestion absorption or utilization of essential vitamins or by factors that increase their requirement destruction or excretion [236 429 430]

CONDITIONED VITAMIN DEFICIENCY

I FACTORS INTERFERING WITH INGESTION

1 Personal
    Economic ignorance poor food habits food faddism eccentricity alcoholism anxiety
2 Gastro intestinal disease
    Anorexia due to alcohol anæsthesia post operative conditions infectious disease and visceral pain
    Dysphagia
    Dyspepsia
    Nutritionally inadequate therapeutic diets as in
        (a) Gastro enteritis
        (b) Cholecystitis and cholelithiasis
        (c) Ulcerative colitis
        (d) Peptic ulcer
        (e) Obesity treatment
        (f) Chronic renal hepatic and cardiac disease
        (g) Carcinoma of stomach and œsophagus cardiospasm
        (h) Intestinal obstruction
3 Food allergy
4 Mental disorders such as
    Neurasthenia
    Neurosis
    Psychoneurosis
    Psychosis
    Anorexia nervosa
    Migraine
5 Operations and anæsthesia
6 Loss of teeth
7 Heart failure (anorexia nausea and vomiting due to visceral congestion)
8 Parenteral administration of nutrients e g saline glucose amino acids
9 Pulmonary disease (anorexia vomiting due to cough)
10 Toxæmia of pregnancy (nausea and vomiting)
11 Neurological diseases interfering with feeding chewing and swallowing

II FACTORS INTERFERING WITH ABSORPTION

1 Diarrhœal diseases
    Ulcerative and mucous colitis
    Dysentery and intestinal parasites
    Intestinal tuberculosis
    Sprue
2 Gastro intestinal diseases associated with hypermotility or reduction of absorbing surfaces e g carcinoma sprue colitis
3 Gastro intestinal and external fistula strictures
4 Short circuiting operations on the bowel
5 Vomiting
6 Achlorhydria
7 Biliary disease especially obstructive jaundice
8 Therapy—liquid paraffin colloidal adsorbents and cathartics

III FACTORS INTERFERING WITH UTILIZATION

1 Hepatic dysfunction
2 Diabetes
3 Alcoholism
4 Hypothyroidism
5 Malignancy
6 Therapy—sulphonamide and other drugs, radiation therapy

IV FACTORS INCREASING REQUIREMENT

1 Abnormal activity ?—e g prolonged strenuous physical exertion
    Delirium
    Mania
2 Fever
3 Hyperthyroidism
4 Pregnancy and lactation
5 Abnormal environmental factors
    Excessive temperature, as in tropics and certain industries
6 Therapy increasing metabolic rate, such as thyroid, insulin, feve therapy, parenteral dextrose, high carbohydrate diets
7 Anoxia

V FACTORS CAUSING DESTRUCTION OF VITAMINS

1 Achlorhydria ?
2 Lead poisoning ? Trinitrotoluene poisoning ?
3 Therapy with
    Alkalis
    Sulphonamides, sulphones, antibiotics
    Arsenicals
4 Antivitamins, e g thiaminase

VI FACTORS INCREASING EXCRETION

1 Polyuria as in
    Diabetes mellitus
    Diabetes insipidus
    Nephritis
    Diuresis induced by drugs
2 Lactation
3 Excessive perspiration ?
4 Therapy excessive fluid intake—for urinary and other infections

VII FACTORS CAUSING DIMINISHED INTESTINAL SYNTHESIS

1 Sulphonamides
2 Antibiotics, e g penicillin, terramycin, aureomycin, chloramphenicol

Not al
ments of
and temp
aneurine

I **Factors Interfering with Ingestion** An inadequate intake of aneurine may result from poverty, ignorance of what constitutes a balanced diet poor food habits food fads and eccentricity We have seen patients with poor

describes a girl with ...

Gastro intestinal disease, especially if associated with anorexia dysphagia

dyspepsia, pain or vomiting, such as occurs in nervous dyspepsia, peptic

r ulcerative colitis, are noted for
atient, either because of pain or
diet The nervous tense woman
with " nervous dyspepsia " or an " irritable colon " associated with abdominal pain who gradually reduces her diet to tea and toast, and who subsequently develops a sore tongue and signs of peripheral neuritis is also well known

loss of weight, stomatitis, peripheral neuritis and even pellagrous lesions following strict dietary treatment for non organic digestive symptoms and for the relief of hay fever and asthma

It is clear that many therapeutic diets even if well planned need to be supplemented by vitamins and minerals in which they are deficient Particularly harmful are many of the slimming diets that are published in non medical papers Dried brewers' yeast and yeast extract are useful sources of the B vitamins for incorporation in therapeutic diets Obstructive lesions such as carcinoma of the stomach and œsophagus and intestinal obstruction also limit food and hence vitamin intake

In neuropsychiatric disorders, such as neurasthenia the neuroses, psychoses and anorexia nervosa the patient may have no desire for food In migraine and hyperemesis gravidarum the mere sight of food may induce nausea Anorexia is also associated with alcoholism, anæsthesia post-operative convalescence, infectious disease and visceral pain, and if prolonged may lead to severe vitamin deprivation Alcohol, which contains no vitamins, produces deficiency disease by replacing other food and by causing nausea and vomiting by its irritant action on the stomach, in which it sets up a chronic gastritis Alcoholism therefore produces aneurine deficiency by causing anorexia and by replacing aneurine containing foods Civilized man usually satisfies anorexia not by a limited food intake all round, but by snacks of carbohydrate foods, such as bread, toast and sugared beverages, which increase the relative aneurine requirement Anorexia may also occur in old people, particularly if edentulous and leading a solitary existence Lack of teeth results in the consumption of pappy carbohydrate foods, which if refined are poor in aneurine

In heart failure and pulmonary disease the anorexia nausea and vomiting due to visceral congestion and cough limit the intake of food When saline, glucose and amino acids are given parenterally because the patient cannot take food by mouth there is no aneurine except the small amount stored in the body, to metabolize the glucose Aneurine, nicotinic acid, riboflavine and ascorbic acid should be added to solutions for infusion if nothing is taken by mouth Certain neurological diseases characterized by paralysis of the muscles of deglutition and cardiospasm may also interfere with the neuro muscular mechanism of swallowing Patients with such conditions, as well as those with œsophageal stricture or carcinoma, or obstructive lesions in the gastro intestinal tract, e g pyloric stenosis cardiospasm and carcinoma of the stomach may fail to receive sufficient food by the oral route and suffer from deficiency disease

II Factors Interferi--
absorption of food play
disease, even if the int.
diseases, such as colitis (ulcerative and mucous), dysentery, intestinal parasitism [622], intestinal tuberculosis, sprue and pellagra, absorption of food and hence vitamins is impaired because it is rushed through the intestinal tract leaving little time for digestion, solution and absorption [430, 682] The

absorbing surface of the gut may also be impaired as in chronic ulcerative colitis and sprue and the internal secretions may be so altered that absorption is imperfect Absorption may also be modified by alkalis adsorbents and lubricants (liquid paraffin) used in the treatment of these and other gastro-intestinal diseases The water soluble vitamins are adsorbed by such substances as aluminium hydroxide (e g Aludrox) fuller s earth and magnesium trisilicate [249] and liquid paraffin dissolves out the fat soluble vitamins A D E and K which are thus lost to the body Medicinal charcoal taken for therapeutic reasons removes considerable amounts of aneurine and riboflavine [592] It is claimed that achlorhydria may impair the absorption of aneurine [44 237] although this has been denied [55] Vomiting which is a common symptom of gastro intestinal disease interferes with the absorption of food and if unrelieved may precipitate aneurine deficiency Achlorhydria may limit the absorption of some of the B vitamins [583 586]

Obstructive gastro intestinal lesions at or below the level of the stomach may interfere with the absorption of food Thus a malfunctioning gastro enteric anastomosis may obstruct the stomach and duodenum Obstructive lesions of the small intestine have long been known to be associated with an anemia and a clinical picture simulating that of pernicious anemia and sprue

Fistulæ e g gastro colic which short circuit the small intestine wholly or in part are causes albeit uncommon of deficiency diseases In spite of an adequate food intake so little may reach the small intestine that severe loss of weight and malnutrition may result Syndromes resembling those of sprue and beriberi have been reported in patients with such fistulæ [238] Short circuiting operations of the gastro intestinal tract e g gastrectomy have long been known to produce macrocytic anæmia and there is some evidence that other deficiency conditions may be produced Vitamin B deficiency may occur in ten per cent of patients submitted to gastrectomy according to Welbourne Hughes and Wells [701] External intestinal fistulæ are uncommon but they have been observed associated with deficiency disease

Liver disease may lead to interference with the absorption of fat soluble vitamins (A D E and K) and possibly with the intestinal absorption of aneurine [720]

From studies on sprue patients Frazer [583] has advanced a new mechanism of vit [illegible] of sprue cases [illegible] the upper [illegible] ost Frazer suggests that in the exacerbations of sprue and possibly in other conditions such as pellagra pernicious anemia and nutritional macrocytic anæmia there is a competition for essential nutrients between the host and bacteria in the absorption area of the intestine which does not harbour bacteria in the healthy subject This view is supported by the observation that in sprue parenteral administration of the appropriate vitamin often produces immediate relief of vitamin deficiency syndromes without affecting fat absorption

III Factors Interfering with Utilization Evidence for the existence of factors interfering with the utilization of vitamins is largely circumstantial It is know that certain vitamins cannot be utilized by the body as such Thus carotene must be converted to vitamin A aneurine to cocarboxylase nicotinic acid to codehydrogenases I and II and riboflavine to flavoprotein The liver is considered to be the principal organ in which some of these conversions occur

Cirrhosis and other diseases of the liver are believed to inhibit the utilization of aneurine the excretion of which is lowered in hepatic disease The high frequency of symptoms of aneurine deficiency in alcoholics is well known and it is possible that hepatic dysfunction plays a part It is believed by some

that diabetes interferes with the utilization of aneurine although the evidence for this is conflicting In malignant disease there appears to be a general vitamin deficiency

Therapy with some sulphonamides and other drugs may interfere with the utilization of the B vitamins It is known that deficiency symptoms can be produced by administering sulphonamide drugs to animals although this may be due to the inhibition of the intestinal synthesis of some of the B factors (pp 127 136) Sulphadiazine may actually have an aneurine sparing action (p 198) Sulphapyridine inhibits the activity of nicotinic acid in the experimental animal [664] but there is no evidence that it does in the pellagrin [429] Deficiency symptoms have been produced in man following the administration of sulphonamides sulphones and antibiotics such as penicillin streptomycin chloramphenicol and aureomycin [790] Radiation sickness has been attributed to failure of co enzyme formation from aneurine and nicotinic acid

IV **Factors Increasing Requirement** It has already been stated that the aneurine requirement is proportional to the metabolic rate (p 210) Fever increases the basal metabolism by 7 2 per cent for each degree Fahrenheit while strenuous physical exertion may increase it as much as fifteen times It would be expected that the pyrexial patient requires more aneurine than normal The occurrence of aneurine deficiency conditioned by fever hyper thyroidism (p 210) pregnancy and lactation (p 210) is well recognized Johnson and his co workers [818] state there there is an increased requirement of the B vitamins produced by moderately strenuous physical activity in farmers soldiers and other workers On a consumption of 4 000 to 5 000

t in the

Keys

results

(p 206) It is therefore debatable whether an increased requirement is to be expected in occupations associated with prolonged strenuous physical work and in mental patients with delirium or mania showing an increase in psychomotor activity Outbreaks of deficiency diseases such as pellagra were once common in mental hospitals Excessively high environmental temperatures such as might be experienced in the tropics and in certain industries may increase the

lism various forms of therapy may produce

ieurine Drugs such as thyroid thyroxine

and fever therapy increase metabolism and hence the need for aneurine Parenteral administration of glucose insulin therapy and high carbohydrate diets do not increase total metabolism but increase the number of non fat calories consumed and therefore the requirement of aneurine The long continued administration of glucose to patients on a poor diet may precipitate defi

way over a pro

given as well

disease beriberi due to this cause has in fact been reported [819]

V **Factors causing Destruction of Vitamins** Melnick Robinson and Field [44] have shown that aneurine is stable in gastric juice between a range of pH 1 5 to 8 The presence of antacids bile or pancreatic juice destroys aneurine

its excretio

with the d

(pp 421

inactivate aneurine particularly raw fish and meat This factor thiaminase (p 228) is unlikely to be present in any quantity in most human diets as meat and fish are usually cooked It has been reported however that fifty per cent of the dietary aneurine in man may be destroyed by diets containing raw clams [246] An anti aneurine factor is also stated to be present in

certain cereals, legumes and oil seeds [250], and the administration of live yeast withdraws aneurine from the gut

VI **Factors Increasing Excretion** The possibility of the washing out of the body of the water soluble vitamins must be considered in conditions associated with polyuria, such as diabetes mellitus and diabetes insipidus As Cowgill and his collaborators [224] have shown, excessive diuresis produced by forcing fluids can bring on the symptoms of aneurine deficiency The forcing of fluids over a long period of time as a therapeutic measure in general infections and infections of the urinary tract, and the loss of fluid by diuresis in œdema may precipitate a deficiency The excretion of aneurine is considerably increased by the administration of mercurial diuretics such as mercurophylline [54] Lactation also results in a loss of aneurine from the body (p 210), and is notorious in the East as a factor in the production of beriberi Excessive perspiration has been said to result in appreciable losses of aneurine from the body, but this has not been confirmed (p 203) In any case, loss of aneurine by this route would only be of significance under tropical conditions or in subjects doing heavy manual work in a hot environment

Biochemically Induced Aneurine Deficiency It has been recognized that a compound may so structurally resemble a vitamin or growth factor that it will be adsorbed in an enzyme system containing the vitamin in place of the latter The difference, however, is sufficient to prevent the cycle of chemical reactions occurring The enzyme system is thus "blocked," e g sulphanilamide blocks the system containing *p* aminobenzoic acid (p 135) and pantoyltaurine blocks that containing pantothenic acid (p 120) If the enzyme system of which the vitamin is a component is blocked, the latter is not available for growth and development of the organism Pantothenic acid and *p* aminobenzoic deficiencies can be produced in animals by feeding the appropriate inactivating agent Woolley and White [905] have produced aneurine deficiency in mice by the addition of a thiamin analogue, known as

pyridine If sufficiently

covery occurs A com

nimals fed large amounts

l to develop deficiency

is increased sufficiently

Other aneurine analogues that produce deficiency symptoms by a similar

thiamin [259] and butyl

iochemical inhibitor may

disease, as they circum

vent the use of synthetic or purified diets, which are laborious to prepare and unappetizing to eat

Another mechanism is involved in the destruction of aneurine by enzymes present in certain animals and plants The paralysis that occurs in foxes on fox ranches as a result of feeding them raw fish (Chastek paralysis) is due to aneurine deficiency caused by an aneurine destroying enzyme, thiaminase [829] This disease is fatal if untreated, it responds to treatment with aneurine or withdrawal of ra

into its pyrimidine and thiazo

moiety with an amine [820],

salts [252] Thiaminase can cause aneurine deficiency symptoms in chicks cats and pigeons as well as foxes, and is in fact a useful tool for the production of aneurine deficiency The importance of thiaminase in human nutrition is not known In countries where raw, partly cooked or smoked fish constitutes a major constituent of the diet it may be a factor contributing to aneurine deficency Clams and salted herring have a very powerful thiaminase that may destroy fifty to sixty per cent of the dietary aneurine, oysters and smoked salmon are innocent in this respect [246]

A plant factor from bracken fern, enzymic in nature, has been isolated which causes signs of aneurine deficiency and is responsible for disease in

horses and cattle feeding on fern pasture [256, 263, 651] Other aneurine inhibitors have been described in grain [250], raw mutton [258] and the plant equisetum (horsetail) [770]

Nervous Lesions in Aneurine Deficiency. In animals suffering from acute aneurine deficiency Wallerian degeneration of peripheral nerve fibres occurs [289], and degenerative changes have also been described in the anterior and posterior nerve roots, tracts of the spinal cord, the medulla, pons midbrain and internal capsule. In the deficient pigeon the first neuronal histological change is degeneration of the distal part of the axon, which proceeds towards the cell body, which becomes sclerosed; the large nerve fibres degenerate first [732]. If the deficiency is chronic, degeneration of the vestibular nerves results, which is often associated with cerebellar ataxia, and sometime degeneration of the cell bodies and peripheral fibres of the third and fourth cranial nerves There is a progressive increase in the amplitude of the potentials, up to about three times normal, in the encephalogram of pigeons with aneurine deficiency In the final stages there is a marked *slowing down and depression of the brain potentials* The *electroencephalo* gram returns to normal in a few hours if aneurine is given [821] The neurological manifestations of aneurine deficiency in the pigeon are accompanied first by impaired function and then by degeneration of primary neurons of the proprioceptive nervous system and the central terminations of the optic nerves, after prolonged deficiency the efferent nervous system becomes affected. A number of c ... en aneurine is given, but severely injured on ... Increased amounts of alkaline phosphata ... ells, and a diminution of acid phosphatase in the axis cylinders [738] If aneurine deficiency is continued long enough in animals the neurological lesions are irreversible [822] The vascular changes in the brains of aneurine deficient animals have been studied by Prados and Swank [732], who found that hæmorrhages occurred in the brain accompanied by perivascular sclerosis and interstitial cell proliferation The hæmorrhages were preceded by vasodilatation and were first perivascular and later infiltrating. The lesions were accompanied or preceded by degenerative changes in the neighbouring neurons and swelling or hypertrophy of

The dura of aneurine deficient rats s ... dilatation of the vessels and hyperæmia ... results of aneurine deficiency on the nervous system of the cat After an initial period of anorexia the animal suffers from tonic convulsive seizures and disturbances of postural mechanisms, such as impairment of labyrinthine righting reactions, the vestibulo ocular reflex and the pupillary light reflex. Dysfunction of the cerebellum is suggested by asynergia, ataxia and dysmetria The peripheral nerves in the cat are not affected [269]

In the rhesus monkey changes in the peripheral nerves are absent, even with severe aneurine deficiency [180], but changes in the brain similar to those seen in Wernicke's encephalopathy in man have been reported by Rinehart [180] Bilateral areas of degeneration occur in the corpus striatum, globus pallidus, substantia nigra, mamillary bodies, corpora quadrigemina, cerebellar cortex and nuclei of the third, sixth, eighth and tenth cranial nerves Associated with these lesions are profound weakness, ataxia and occasional focal s

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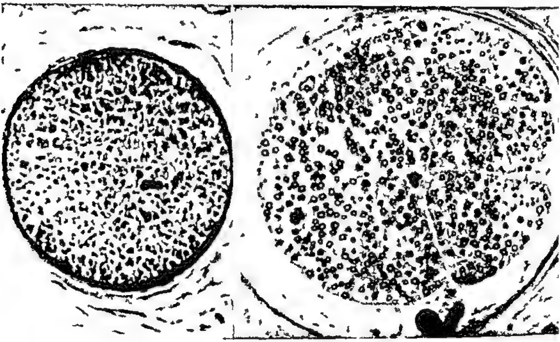
and hyperæsthesia of the soles are the earliest symptoms Such symptoms, which are bilateral, can be observed before the onset of typical neuritic beriberi, in which diminished reflexes and muscular paralysis and atrophy

make their appearance Vibration and
jerks may disappear The terminal po
and therefore the symptoms are more p
extremities chiefly the lower in which the sciatic nerve and its branches suffer Histological changes (Fig 73) are of late onset and may be sti seen after symptomatic improvement The first stages of recovery from th neuritis may be rapid but many weeks may elapse before the complete us of the legs is regained The slow improvement in severe neuritic cases unde treatment is undoubtedly due to the long time taken for the remyelinizatio of the nerve fibres

In moderately advanced cases pain along trunks and along intercosta nerves may be complained of Dropped foot and wrist and muscular atrophy especially of the thigh may result and then the upper extremities may b



Figs

74 is a section across the same nerve of a normal person as ... to ... *myelin sheath count is c 840 per sq mm* (Osmic acid stain × 150)

affected As the sensory changes advance pain and numbness increase Finally the muscular and sensory changes
may occur and the patient becomes bed
may produce paresis of the vocal cords
the abducent may be affected as in Wernicke s encephalopathy (p 231) A neuritis of the tenth nerve or its laryngeal branches leading to changes in the voice have been described Lesions of the optic nerve—diminished visual fields temporal pallor of the disc and primary optic atrophy—have been described in patients suffering from severe vitamin B deficiency although several factors of the vitamin B complex may probably be involved [751] No significant degeneration of the optic nerve is seen however in animals on diets deficient in aneurine or the vitamin B complex [550]

The neurological symptoms that may occur in such diverse conditions as nutritional g ... 262 264] and alcoholic neuritis [266 ... have been attributed to aneurine defic ... a deficiency of aneurine

in these conditions, if a vitamin deficiency exists it is likely to be a multiple one Gastro intestinal conditions such as diarrhœa, colitis, sprue and pyloric stenosis associated with vomiting are associated with multiple avitaminosis (p 225) Lewy [418] noted that the degree of peripheral nerve change in pregnant women on defective diets as indicated by chronaximetric examination, coincided with the severity of the condition Improvement followed the administration of vitamin B If the diet contains adequate aneurine, pregnancy neuritis does not occur [270]

chsler [277] that diabetic neuritis might was later claimed that the administra Needles [281], however, examined the diets of a number of diabetics and concluded that they contained an adequate amount of aneurine

It has been suggested that alcoholic neuritis results from vitamin deficiency (p 225) The alcoholic buys alcohol rather than food and suffers from anorexia and gastritis These limit his food and vitamin intake and absorption This is the most likely explanation of a deficiency rather than increased needs of aneurine due to the alcohol

Some cases of nutritional neuropathy are not due to aneurine deficiency, as shown by the work of Grande and Jimenez [824] who investigated cases of neuropathy during the Spanish Civil War They found that lactic acid was metabolized normally and therefore concluded that the neuropathies they observed were not ætiologically related to aneurine The diet in Spain consisted largely of bread lentils, rice and soup and was not alarmingly low in aneurine Pellagra was common, but not beriberi These neuropathies were not cured by aneurine, nicotinic acid or vitamin A, but responded to treatment with yeast

Wernicke's Encephalopathy The disease known as superior hæmorrhagic polioencephalitis, or Wernicke's encephalopathy, was originally described in 1881 by Wernicke and is characterized by paralysis of the eye muscles, a reeling gate and disturbances of consciousness which usually terminate in fatal com

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colliculi and the floor of the fourth ventricle (Figs 75–79) Common clinical

loss of memory for recent events Less common are loss of visual acuity, fundal hæmorrhages, cranial nerve pulses, incontinence and ataxia Wernicke's encephalopathy is closely related clinically to Korsakoff's syndrome (p 252) and is often associated with alcoholism, although in twelve cases reported by Campbell and Biggart [284] only one was alcoholic

The condition has been more recently described by Campbell and Biggart [284] nan [545], Wortis *et al* [743] Boles [834] and Cruickshank ns, but is mostly associated with conditioned nutritional failure, e g in alcoholism, liver d

d

ir

The disease is generally considered to be due to acute aneurine deficiency, because it is always associated with a low intake or diminished absorption or utilization of the latter, and it responds to treatment with the vitamin Experimental support for this view is afforded by the observations of Prickett [285] who observed foci of congestion, hæmorrhage and degeneration in the pons, medulla and cerebellum of aneurine deficient rats, and by Alexander and his co workers [286] who produced similar lesions in aneurine

Fig 75 Field from lesion in corpus mamillare showing vascular dilatation and endothelial hyperplasia Nissl stain × 300

Fig 76 Sub[...] midbrain showing vascular dilatation, [...] walls and cellular proliferation × 65

Fig 77 Field from lesion in corpus mamillare showing fat (dark granules) in proliferated microglia cells Scharlach R and hematoxylin × 250

WERNICKE'S ENCEPHALOPATHY

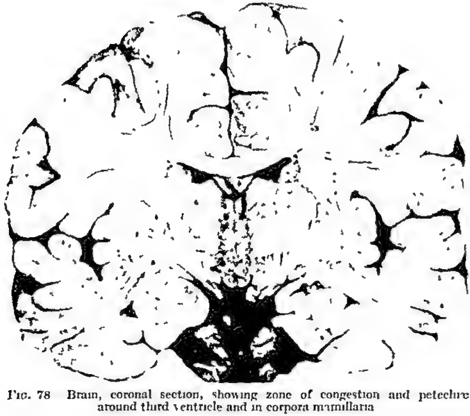

Fig. 78 Brain, coronal section, showing zone of congestion and petechiae around third ventricle and in corpora mamillaria

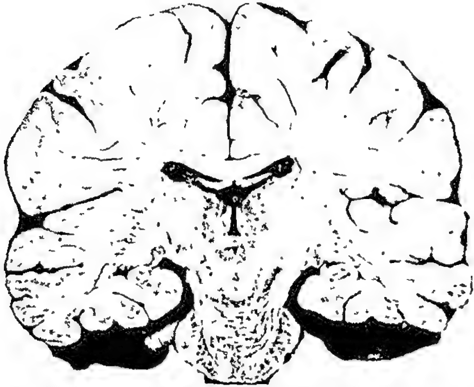

Fig. 79. Brain, coronal section, showing similar zonal lesion around posterior end of third ventricle and upper end of aqueduct

deficient pigeons. Rinehart [180] states that the lesions in the central nervous system of the rhesus monkey kept on an aneurine deficient diet correspond in a general way to Wernicke's encephalopathy, although there are minor differences, such as the relative absence of vascular changes and the severe changes in the corpus striatum (p. 229). The pathology of Chastek paralysis in foxes, produced by feeding them raw fish (p. 228), is similar to that seen in Wernicke's encephalopathy.

The condition responds to treatment with aneurine, although residual manifestations such as nystagmus, psychotic symptoms and mental deterioration may persist. Severe cases may be fatal in spite of treatment. Jolliffe and his co-workers [743] and Campbell and Biggart [284] consider that the condition is not due solely to aneurine deficiency and that nicotinic acid deficiency may play a part (see p. 369). The dramatic improvement often occurring after injecting aneurine suggests that Wernicke's encephalopathy is largely due to deficiency of this vitamin (p. 253).

Psychological Manifestations of Aneurine Deficiency. By maintaining volunteers on diets deficient in aneurine, deficiency symptoms have been produced. Mental symptoms resembling those of neurasthenia are common—intolerance of noise, peculiar sensations in the head, inability to concentrate, inattention to details, memory defects, irritability, "nervousness," anxiety, depression and insomnia [327, 331, 697, 713, 715, 826]. The individual becomes depressed, uncooperative, apprehensive, irritable and quarrelsome and suffers from lack of interest and ambition. In tests made by Brozek, Guetzkow and Keys [282] and by Henderson *et al.* [311] the Minnesota Multiphasic Personality Inventory showed significant changes in the scores on the three psychoneurotic scales—depression, hysteria and hypochondriasis. The Rorschach findings showed loss of spontaneity and an increase in tension. These workers consider that the neurasthenic symptoms of early pellagra (p. 360) are manifestations of aneurine deficiency. Horwitt and his colleagues [283] kept patients on diets containing restricted amounts of aneurine (0.2 to 0.4 mg. daily) for a period of three years. They noted an increased psychotic tendency, decrease in vibration sense and a rise in blood lactate and pyruvate.

Wernicke's encephalopathy is discussed on p. 231.

Cardiac Lesions in Aneurine Deficiency. In the experimental animal aneurine deficiency produces cardiac lesions. In the rat there is marked bradycardia, ectopic beats, widening of the PR interval, notched P waves, changes in the T waves and ST segments, dilatation of the right auricle and post-mortem necrosis of the cardiac muscle fibres with cellular infiltration [292, 294, 307, 896]. In the pig aneurine deficiency causes cardiac dilatation, myocardial necrosis, bradycardia, disturbances of AV conduction, heart block and necrosis of the Purkinje cells [292, 302, 830]. Swank, Porter and Yeomans [303] noted dilatation of the auricle of the aneurine deficient dog.

Cardiac lesions occur in man as a result of aneurine deficiency in the absence of symptoms of frank beriberi. They are often referred to as the "beriberi heart" and, although relatively common in the United States as a result of alcoholism and gross dietary deficiency, the condition is sufficiently rare in Britain to justify the publication of single cases [689–695]. Of 120 cases studied by Weiss and Wilkins [242] in America the commonest causes were chronic alcoholism, poor dietary history, drug addiction and the vomiting of pregnancy. The following signs and symptoms are commonly seen: tachycardia, arrhythmia, dyspnœa on exertion, reduced vital capacity, palpitations, cardiac murmurs, a bounding peripheral pulse, distended veins and sometimes œdema. The skin may be flushed and warm due to peripheral vasodilatation and the peripheral blood flow may be increased. These manifestations have been disputed by Roth, Williams and Sheard [927], whose observations, however, were made on subjects suffering from an induced aneurine deficiency. There is often cardiac enlargement, an increase in

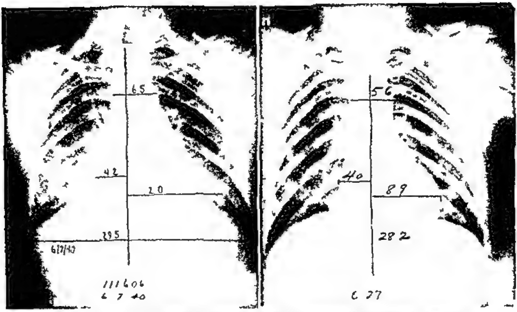

FIG 80 X ray of Patient suffering from Aneurine Deficiency before and twenty days after Treatment The measurements are in centimetres The patient was an alcoholic who rarely ate more than one meal daily and ... nd legs and soles ... was enlarged on ... The first X ray ... ond X ray taken ... shows return of heart shadow to normal size The oedema and systolic murmur disappeared (Drs Porter and Downs case)

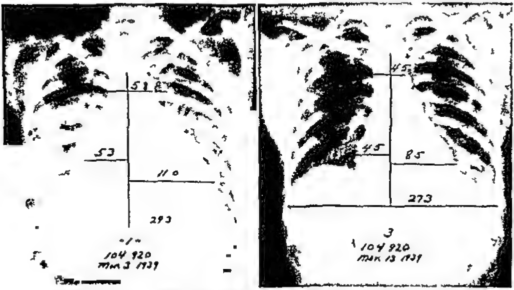

FIG 81 X ray of Patient suffering from Aneurine Deficiency before and after ten days Treatment Measurements in centimetres The patient was an alcoholic taxi driver living largely on snacks who had swollen and painful legs and tingling of the fingers The condition became worse and the patient had an attack of delirium tremens with tremors over activity hallucinations and disorientation On examination the heart was enlarged knee and ankle jerks were absent muscle weakness and tenderness were present in the arms and legs which showed pitting oedema There was a high pitched blowing systolic murmur gallop rhythm and enlarged liver The first X ray shows enlargement of the heart to right and left The second X ray showing return of the heart to normal size was taken ten days later after daily injections of 12 mg aneurine and yeast orally

ECG IN ANEURINE DEFICIENCY

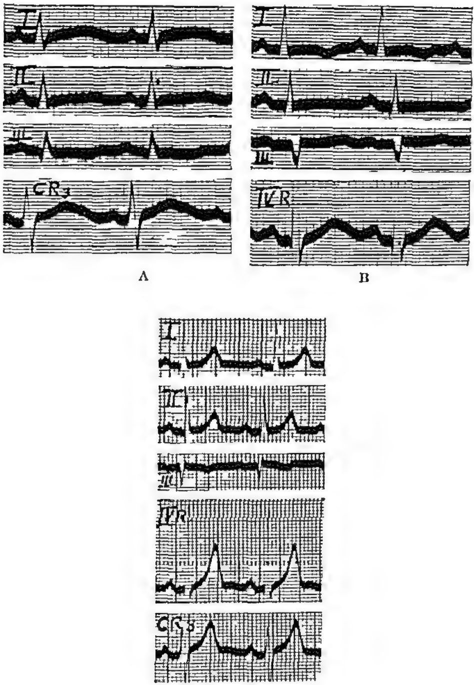

FIG 82 Electrocardiogram from a Case of Circulatory Failure due to Aneurine Deficiency The [illegible] —shortness of breath œdem[illegible] enlarged liver and heart [illegible] eflections upwards in all lim[illegible] l upright, T_2 almost absent and T_3 inverted and QT inter[illegible] ased (0 49 sec) The second E C G was taken twenty days after treatment with aneurine (20 mg daily by mouth and 7 injections of 25 mg) R_1 has [illegible] rted T_3 is [illegible] T interval [illegible] coronary [illegible] ws normal tracings for all leads and normal duration of electrical systole (QT = 0 42 sec) *Time markings in A and B are 1/25 sec and 1/25 and 1/5 sec in C*

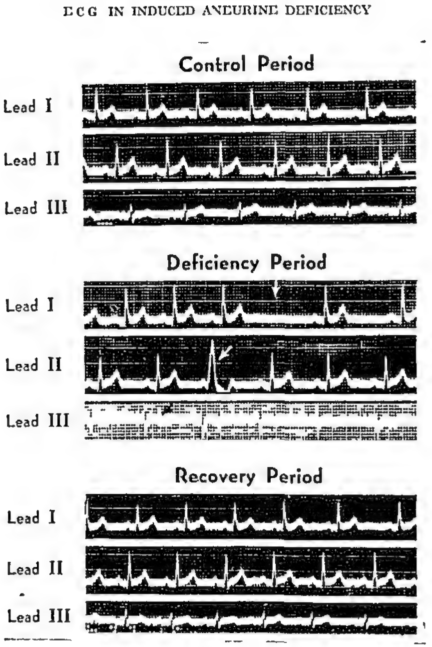

FIG 83 Electrocardiogram in a Case of induced Aneurine Deficiency The subject was placed on a diet of 0 17 mg aneurine daily and an aneurine calorie ratio of 1. Changes in the ECG occurred on the eleventh day after commencing the diet They include sinus arrhythmia, sinus arrest, *change in deviation of electrical axis and inversion of T_3* After administering adequate aneurine the ECG was restored to normal on the fifth day of treatment

venous pressure and changes in the electrocardiogram although these are non specific Auriculo ventricular and intraventricular block have been reported [290] Repeated attacks of aneurine deficiency may leave a residuum of permanent damage in the heart The principal changes seen in the electrocardiogram are tachycardia flattening and inversion of the T waves changes in the PR interval and increased QT interval increase in the length of systole and low voltage complexes

Cardiac lesions due to aneurine deficiency are comparatively rare outside the tropics They may occur as a result of restricted diets deficient assimilation or any of the factors operating to produce aneurine deficiency described on p 223 The recognition of cardiac dysfunction due to aneurine deficiency is based on the following diagnostic criteria a history of nutritional deficiency due to deficient intake or 'conditioned' (p 223), tachycardia not due to known causes such as hyperthyroidism palpitations venous congestion right sided enlargement of the heart, œdema not in keeping with the cardiac and renal picture and the plasma protein level (the latter may be lowered in general malnutrition) and changes in the electrocardiogram According to Porter and Downs [831] the cardiac output and oxygen consumption are increased and the arterio venous oxygen difference is decreased The blood pyruvate (p 242) and blood sugar are increased and there is a higher pyruvate level in arterial blood [293] A high blood pyruvate is of no diagnostic significance [253] Aneurine is of no value in the treatment of cardiac conditions not due to aneurine deficiency This has been convincingly shown by Weiss [629] and by Sutton Friedemann and Simpson [253] Heart disease of nutritional origin not due to aneurine deficiency has been described [686]

Aneurine Deficiency and Gastro intestinal Symptoms One of the earliest manifestations of aneurine deficiency in animals is anorexia which is rapidly relieved by administering the vitamin Other gastro intestinal manifestations that have been observed in aneurine deficient animals are vomiting loss of intestinal tonus [539] and impaired absorption of glucose from the intestine [20] Radiological studies have shown that gastro intestinal hypotonicity dilatation and stasis follow a pure aneurine deficiency in the rat [736 737] The production of peptic ulcers has also been described [306 312 313]

In man as in the animal anorexia is an early symptom of aneurine deficiency Experiments on induced aneurine deficiency in man have often had to be terminated because of this Constipation [327] hypo and achlorhydria [308 377] impaired gastric secretion slow gastric emptying time and sluggish intestinal motility [304 327] have also been reported although Maxwell and his co workers [879] failed to observe any significant radiological abnormalities in patients with low blood aneurine levels Abdominal discomfort heartburn and constipation occurred in a volunteer on a diet deficient in the vitamin B complex [331 713] In infantile beriberi gastro intestinal symptoms are common they include anorexia regurgitation abdominal distension tenderness vomiting and constipation

Aneurine is often prescribed for the treatment of gastro intestinal symptoms such as anorexia and constipation and is a common constituent of proprietary tonics There is no therapeutic justification for this unless the patient is known to be suffering from aneurine deficiency In the rhesus monkey aneurine has no laxative effect [833]

Leithauser [589] describes nine cases of abdominal distension severe enough to involve laparotomy in four owing to a mistaken diagnosis of appendicitis or carcinoma The patients were alcoholics on restricted diets Four were treated with aneurine with apparent relief of distension

Experimental Aneurine Deficiency in Man Many studies have been made mainly in America on the effects of a partial deficiency of aneurine in human subjects [92 93 282 283 311 315 327 328 331 696 697 713 715 760 793 826 890 945] The studies were carried on for varying periods ranging from

a few weeks to three years (Horwitt *et al* [283]) the daily aneurine intake varied from 0 15 to 0 5 mg The deficiency symptoms varied widely depending on the level of aneurine intake and the duration of the test The outstanding symptoms are anorexia fatigue and polyneuropathy The following manifestations have been recorded

General Symptoms Fatigue anorexia loss of weight dyspnœa on slight exertion photophobia backache deterioration in physical fitness thinning and loss of elasticity of skin frequency of micturition a decreased sense of well being and anæmia The anæmia is characterized by an increase in the mean corpuscular volume and hæmoglobin macrocytosis polychromasia and poikilocytosis [715] The early stage of deficiency simulates neurasthenia the later one anorexia nervosa

præcordial pain precipitated by effort sinus arrhythmia on exertion pallor and a feeling of coldness The heart is not enlarged and according to Williams and his co workers [327] there is no œdema This is recorded by Elsom and his collaborators [331 713] although their subjects were suffering from a vitamin B comple

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disturbance as evidenced by skin temperature measurements and determination of rates of cooling and warming of body tissue [514] this is in contrast to the moist warm skin of beriberi (p 219)

Respiratory Dyspnœa on effort reduction in respiratory efficiency

Mental Changes Listlessness apathy dulling of interest and ambition inability to concentrate confusion of thought and memory loss of memory depression paranoid tendencies irritability quarrelsomeness inattention to details headache tenseness insomnia impairment of foresight and judgment hysteria and hypochondriasis The results of applying personality tests are described on p 234

Nervous System Depressed tendon reflexes increased sensitivity to painful stimuli vague paresthesiæ polyneuropathy muscle cramps tenderness of calf muscles diminished vibration sense and diminution of electrical irritability of peripheral nerves

These experiments on human volunteers do show that aneurine can directly or indirectly lead to disturbances of function of the peripheral nerves of the lower limbs If the deficiency is sufficiently prolonged it leads to a lower motor neurone type of lesion which judged by the slow response to treatment is morphological and not readily reversible

Psychomotor and Performance Tests In subjects kept on a diet deficient in aneurine there is diminishec

tests [317 3?0] and increased

ance and deterioration in

hearing or speed [315 318 69 ... In some of the tests made the subjects were placed on diets deficient in the other B vitamins as well so that in these cases it is difficult to ascribe any of the results obtained to the deficiency of one vitamin Reaction time is diminished on low aneurine intakes e g 0 20 mg daily [319]

Laboratory Tests There is diminished excretion of aneurine a rise in

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same authors [793] and by others [283, 315] showed an increase in blood lactate and pyruvate at rest, during work and after the administration of glucose.

Diagnosis, Treatment and Prevention of Aneurine Deficiency. The symptomatology of early aneurine deficiency resembles that of neurasthenia. Diagnosis depends on a careful dietary history and a complete examination of the nervous and cardiovascular symptoms. The following manifestations are of significance. Tenderness of calf muscles, hyperæsthesia, muscle cramps and weakness, all of which appear before the more serious neurological complications; lost or diminished ankle and knee jerks and vibration sense may be observed. Anorexia, fatigue, dyspnœa on exertion, loss of weight and irritability are early symptoms. Factors predisposing to nutritional deficiency should be looked for (p. 223). A therapeutic test should be performed in suspected cases and, if there is no improvement after a few weeks' treatment, another diagnosis considered, although advanced cases may require many weeks' treatment before any improvement occurs. The value of laboratory tests in the diagnosis of aneurine deficiency is discussed on p. 242.

For the treatment of aneurine deficiency the measures laid down for that of beriberi should be followed (p. 222). The daily administration of 10 mg. of aneurine is adequate, the dose being reduced to 5 mg. as recovery occurs. Since there is likely to be a deficiency of other B vitamins, the diet should be supplemented with sources of the vitamin B complex such as yeast (heat treated), liver by mouth or injection, and the whole grain of cereals. The necessity for giving the whole of the vitamin B complex rather than massive doses of aneurine is stressed by Sydenstricker [767], who has observed that large doses of one vitamin may precipitate symptoms of a deficiency of another, e.g. ariboflavinosis. It has been shown that large doses of aneurine affect the excretion of riboflavine [768]. The rate of regeneration of nerve is about 1 mm. daily, so that in severe cases of aneurine deficiency many months or even a year may be required for complete recovery of some nerves, e.g. the sciatic. In paralysed cases irreversible changes in the nervous system may occur with permanent neurological damage.

For the prevention of aneurine deficiency in areas where it is likely to be endemic the following measures are suggested: whole wheat or other cereal bread should be substituted for white bread and refined cereals. If this is not possible because of the dietary habits and prejudices of the people the "fortification" of bread as practised in America (p. 187) should be considered. It is the height of human folly to extract essential nutrients from natural food, make them synthetically in the laboratory and then add them to the denatured foodstuff. Until the dietary habits of civilized man are reformed such measures may be necessary. The objection to whole-grain bread is one of custom. Infants can be weaned on to it and like it, and they will continue to eat it unless their parents do not; even peptic ulcer patients can tolerate it if it is gradually introduced into their diet.

METHODS USED FOR THE ASSAY OF ANEURINE

Biological Assay [427]. The most widely used biological assay method depends upon the cure of bradycardia in the rat suffering from aneurine deficiency [348–351, 379–381]. The assay is time consuming—it takes about fifty days to deplete the animals and three days to perform the test—and involves the use of a large number of animals. The catatorulin test of Peters [353, 354] depends on the fact that the oxygen consumption by brain tissue of avitaminotic pigeons is low and is stimulated by minute amounts of aneurine, e.g. 0·2 microgram.

Microbiological Assay. An assay method based on the use of the mould *Phycomyces blakesleeanus* and devised by Schöpfer [382] has been used by a number of workers for estimating aneurine in body fluids [356–359, 383, 638, 839, 840, 909, 948]. The method is based on the observation that the extent

of the growth of this mould is proportional within certain limits to the concentration of aneurine in the medium After inoculating flasks, containing a known amount of aneurine and the test substance respectively, with spores of the mould, the flasks are set aside in the dark at 20° C for four to ten days, when the mycelia of the mould are removed, washed and dried The weights of the mycelia are approximately proportional to the amount of aneurine present

A yeast fermentation method, based on the observation that aneurine increases the rate of production of carbon dioxide by yeast, is widely used [332, 362–365, 526, 852, 903] By the use of a Warburg apparatus aneurine can be assayed on a micro scale of 0 005 to 0 025 micrograms Next to the thiochrome method, this is probably the most widely used and is comparable in accuracy

Assay methods involving the growth of bacteria, such as *Staphylococcus aureus* [361] *Streptococcus salivarius* [853] and *Lactobacillus fermentum* [604, 605, 946] have also been used These organisms are very sensitive to the presence of minute amounts of aneurine

Thiochrome Method Aneurine is oxidized quantitatively in alkaline solution to thiochrome (p 185) which fluoresces under ultra violet light and can be estimated fluorimetrically by comparison with a standard solution This method of estimating aneurine was devised by Jansen [385] and adapted by a number of workers [386–402, 455–459, 599, 703, 841–850, 893, 949, 952] It can be used for estimating free and combined aneurine in blood, urine and other body fluids and in foodstuffs A micromethod capable of detecting 0 05 microgram of aneurine with an accuracy of ± three per cent has been devised [849] Aspirin, salicylates, quinine and related drugs interfere with the thiochrome fluorescence [457, 843, 893]

The following steps are carried out in estimating aneurine by the thio chrome method (*a*) the aneurine and cocarboxylase in the material under examination are extracted with dilute acid , (*b*) any cocarboxylase present is digested by clarase or other preparation rich in phosphatase , (*c*) selective adsorption of the aneurine from (*a*) and (*b*) by " decalso " or similar adsorbing agent , (*d*) elution of the thiamine , (*e*) oxidation of the thiamine to thiochrome by alkaline potassium ferricyanide , (*f*) extraction of the thiochrome with isobutyl alcohol , (*g*) measurement of the fluorescence produced by irradiating the thiochrome solution with ultra violet light and comparison with a standard thiochrome solution

Colorimetric Methods When aneurine reacts in alkaline solution with a diazotized aromatic amine a coloured pigment is formed Prebluda and McCollum [366 367] have used this as a method of assaying aneurine The method has been developed by Melnick and Field [368 369] and others [413, 848, 851, 884] The method is quite satisfactory if there is a relatively high concentration of aneurine in the substance examined e g urine

Kinnersley and Peters [371] have developed a test depending upon the formation of an azo dye between aneurine, formaldehyde and diazotized sulphanilic acid

Estimation of Cocarboxylase As much of the aneurine present in biological media is combined as the pyrophosphate or cocarboxylase, the latter must be hydrolysed before estimation This can be done with yeast extract [407], diastase [458] or kidney extract [408] although a phosphatase preparation known as clarase is usually used The normal blood cocarboxylase in an adult varies from 4 5 to 12 micrograms per 100 ml , with an average of 7 micrograms [377, 417] , it is lower in children [86]

LABORATORY METHODS PROPOSED FOR THE DETECTION OF ANEURINE DEFICIENCY

These depend upon the estimation of aneurine or bisulphite binding substances in the blood, urine or muscle, or upon so called " loading " or " saturation " tests

Estimation of Pyruvic Acid and Bisulphite Binding Substances According to some workers there is an increase in the blood and urine pyruvate and bisulphite binding substances in subjects suffering from beriberi and aneurine deficiency (p 240) It has therefore been used as an index of aneurine nutrition [84 87, 154 314 322, 708] A micro method for its estimation has been devised [333] The normal range of blood pyruvate is 0 3 to 1 28 mg per 100 ml [719, 322, 342 344 580] The upper limit of normal is given as 1 3 mg by Wortis and his co workers [719]

An accumulation of pyruvic acid in the blood may be caused by retardation in its breakdown to carbon dioxide and water or by failure of resynthesis to glucose The factors influencing the level of blood pyruvate are so numerous that a rise in the latter, without any other evidence cannot be considered diagnostic of aneurine deficiency Allibone [13] for example has shown that there is a raised blood pyruvate in toxaemic and haemolytic states and others have observed it in patients with pyrexia [86 322 394] heart disease [343] hepatic cirrhosis [628] and a raised basal metabolic rate [719] Allibone [13] and Joiner *et al* [323] have suggested that in any case in which the resting blood pyruvic acid is raised the response of the level to the administration of aneurine might serve as a therapeutic test for aneurine deficiency Normal blood pyruvate values have been found in chronic beriberi [84] and subclinical symptoms of aneurine deficiency have been produced in volunteers without any change in the blood pyruvate [899] Laing and his co workers [577] found no correlation between the blood pyruvate and aneurine intake of a hundred unselected hospital patients, no correlation has been observed between blood aneurine and blood pyruvate levels [342]

While a high blood pyruvate *per se* cannot be considered diagnostic of aneurine deficiency Williams and his co workers [793] claim that a high blood pyruvate after the intravenous administration of glucose (0 4 gm per kilogram of body weight) is of some diagnostic value The highest level is obtained thirty minutes after injection and this is considered the most significant reading If the glucose is given orally (50 gm in 250 ml repeated in 30 minutes) specimens of blood are taken 30 60 and 90 minutes later maximal values are obtained after 60 to 90 minutes It is claimed that in subjects receiving only 0 1 mg of aneurine per 1 000 calories the blood pyruvate rises from 1 2 to 1 5 mg per cent to 2 3 to 2 6 mg A return to normal after administering aneurine is confirmatory It is also stated that in aneurine deficiency there is a delay in the return of blood pyruvate to normal after exercise Taylor and McHenry [662] estimate the level of blood pyruvate three hours after ingestion of 100 grams of glucose

Keys and his co workers [93] reported a change in the lactate pyruvate ratio in the blood of subjects on a restricted intake of the B vitamins Goldsmith [343] studied this ratio in a number of normal subjects under basal conditions is is 9 to 9 3 [580] and is unchanged following the administration of glucose but increased after exercise The ratio is lower than normal in patients with aneurine riboflavine and nicotinic acid deficiency and in heart disease It returns to normal on administering aneurine According to Goldsmith a lactate pyruvate ration of less than 7 is suggestive of aneurine deficiency The test however is clearly not specific

Horwitt and Kreisler [352] have elaborated a method for detecting sub clinical aneurine deficiency based on estimations of blood glucose lactate and pyruvate following the administration of glucose and mild exercise The subject under test is given by mouth 9 ml of twenty per cent glucose per kilogram of body weight after withdrawing a fasting basal blood sample Sixty minutes later a mild exercise test is performed—walking up and down a flight of twenty one steps 19 cm high twice in sixty seconds—and five minutes later a further blood sample taken From the data obtained an *index of carbohydrate metabolism* (CMI) is calculated from the following formula

$$\mathrm{CMI} = \frac{L - \frac{G}{10} + 15P - \frac{G}{10}}{2} - \frac{1}{2}\left(L + 15P - \frac{G}{5}\right)$$

G, L and P are milligrams of glucose, lactic and pyruvic acid respectively in 100 ml blood Normal values for the CMI are below 15, figures above this indicate a deficiency of aneurine

This seems to be the most satisfactory laboratory test for detecting aneurine deficiency so far devised It was based on observations over a period of four years on normal subjects, patients with aneurine deficiency, and subjects in whom aneurine deficiency had been artificially induced over a long period It must be borne in mind that aneurine deficiency is not the only cause of elevated blood pyruvate and lactate levels Cardiovascular and liver disease infections, thyrotoxicosis, excitement, arsenic and phosphorus poisoning, pernicious vomiting of pregnancy [668] and eclampsia may all cause an increase

Estimation of Blood Aneurine Estimation of the blood aneurine has been used as a means of detecting aneurine deficiency Levels of from 2 to 16 micrograms per 100 ml of blood have been reported [356–359, 375, 377, 393 408, 417 839 855 859], and various workers have assumed that levels of less than 3 to 7 micrograms of aneurine per 100 ml of blood are indicative of aneurine deficiency [357, 375 377 417, 859] Sinclair [355] considers values below 4 5 micrograms abnormal and below 2 micrograms as abnormally low Blood levels however reflect only the immediate past intake, and the wide range of values reported in the literature shows how little they can be relied on in assessing the nutritional status of the individual Thus Benson and his co workers [783] observed a range of 4 8 to 12 3 micrograms in 121 children, and twenty two with aneurine deficiency had blood values within what was considered to be the normal range

Estimation of the Urinary Excretion of Aneurine The estimation of aneurine in the urine has also been used as an index of aneurine intake The excretion figures however vary considerably in apparently adequately nourished subjects Values from 20 to 1,200 micrograms a day have been reported [155–158, 165, 167 328, 329, 369, 370 386–389 414, 580, 861, 862] Excretions considered to be indicative of aneurine deficiency have been variously given from 30 to

386 861] Papageorge an

excretion of aneurine as an

specimen is taken in the hour following completion of a twenty four hour period and after an over night fast They consider that the critical level is 4 micrograms values below this suggesting an inadequate aneurine intake Coryell and her associates [630] found that the mean "fasting hour" excretion was 8 4 micrograms in boys and 7 micrograms in girls Salcedo and his co workers [218] in the Philippines using this excretion test in patients with beriberi, were unable to correlate the severity of the disease with the laboratory data and Sastri and his co workers [580] obtained values ranging from 3 4 to 90 4 micrograms on daily intakes of from 0 8 to 1 39 mg

Subjects suffering from beriberi have been known to have an aneurine excretion within what is considered to be normal limits [218] Gifft and Hauck [71] found striking variations in the aneurine excretion of individuals on the same intake and with the same level of metabolism Others have found no evidence of diminished excretion of aneurine in patients suffering from vitamin B deficiency [891]

The use of a test dose has also been advocated in which the excretion is measured after a dose of aneurine is given by mouth or intramuscularly [155, 157, 165, 388, 414 415, 526, 569, 630, 705, 782, 794, 862, 891] We know little

of the factors involved in these tests, such as the metabolism of aneurine and its excretion (*it is not a threshold substance*), and in view of the wide individual variations obtained (from three to fifty five per cent of the dose according to one group of workers [580]) the results of such tests must be accepted with caution Moreover, practically all the workers in this field have based their conclusions on observations that have not been submitted *to statistical examination* Casual comparison of group averages has led to unwarrantable conclusions

These excretion tests have been severely criticized by Mickelsen Caster and Keys [60] who, in a four year study, point out that most investigators have carried out their observations over a short period of time only before in fact equilibrium was established between intake and excretion *which may take as long as six weeks* if the intake is changed They also state that in many cases the accuracy of the methods used for the determination of aneurine is open to question Mickelsen and his co workers placed their subjects on varying aneurine intakes and the same basic diet for periods of twenty four to thirty four weeks The aneurine in the diet was estimated chemically and the total intake adjusted to a known value by supplements of pure aneurine After a month's rest cross over tests were done on the different subjects The daily excretion of aneurine and pyramin (the ' pyrimidine of Pollock, Ellenberg and Dolger [724]) was determined and the results submitted to the analysis of variance The variations in aneurine excretion appeared in a general way to be linearly related to intake but the variations *from person to person and even in the same person were so great* that it was impossible to apply strict analysis of variance to the data One subject might excrete two to three times as much aneurine as another on the same intake The fact that a change in aneurine intake from 1 to 2 mg required six weeks for aneurine excretion to come to equilibrium with the new intake *shows the fallacy of employing the test dose,* load ' *or* saturation tests planned over a twenty four or even forty eight hour period for estimating the nutritional status of the individual If these variations observed by Mickelsen and his co workers occurred in " normal ' subjects under controlled conditions one would expect an even greater variation among patients examined *because of suspected deficiency* disease, with their possible nutritional, metabolic and digestive peculiarities

Pyramin (Pyrimidine) Estimation *Pollock Ellenberg and Dolger* [724] estimated the urinary excretion of pyramin (called by them pyrimidine) which they considered mirrored the body stores of aneurine Mickelsen [illegible]

[illegible]

with a curve approaching a plateau of about 400 micrograms [illegible] high aneurine intakes In the region of normal intakes (1 to 2 mg daily) the *relationship is very close to linear* Mickelsen Caster and Keys state that pyramin excretion values are not subject to large individual differences such [illegible] levels of aneurine intake [illegible] aneurine becomes zero [illegible] y have also been calculated from the aneurine content of human muscle A small amount of muscle is obtained (5 to 15 mg) with a sterile needle from the glutæal region previously anæsthetized with procaine and the aneurine content estimated [860 902, 903]

THERAPEUTIC USES OF ANEURINE

Aneurine is often administered in large doses e g 100 mg Not only is this wasteful since the maximum amount of aneurine that the body can metabolize is 10 mg daily [58] but large doses of one B vitamin can precipitate symptoms of deficiency of another [767] The maximum amount that can be

absorbed by mouth is 5 mg daily [42] There is no necessity to give aneurine parenterally unless impaired absorption is known or suspected It has been given intrathecally [191 396] but such severe reactions including meningeal irritation can occur from this unnecessary procedure that it is not recommended [572 863]

Neurology *Neuritis* Although a deficiency of aneurine can cause neuritis it is by no means certain that the relationship between aneurine and neuritis is a direct one According to Walshe [360] aneurine is only anti neuritic when the carbohydrate intake is high although why neuritis should result from defective carbohydrate metabolism is unknown In aneurine deficiency in animals and in beriberi in man the peripheral nerves undergo

nowledge of the
of them claimed

to obtain beneficial results The work was largely uncontrolled and often based on observations on small numbers of patients most of whom were also treated by rest in bed analgesics and often physiotherapy Criteria of improvement were not laid down Success of treatment was often measured by the subjective reports of the patient by relief from pain and tenderness and by ability to walk or use the limbs From 1936 onwards a spate of enthusiastic reports appeared but further investigations during the last ten years have failed to substantiate these claims Walshe [372] writing in 1945 stated

Though I have sought it for over twenty years I have yet to see the case of polyneuritis acute or chronic that gave a clear and striking response to the administration of the vitamin B complex or thiamin in whatever dosage and by whatever channel Spillane [146] treated over 200 cases of polyneuritis with aneurine for four years during the 1939–45 war the patients being malnourished natives prisoners of war or internees Those treated with aneurine were in bed and hospitalized just as long as those who received only rest and good food and return of muscular power and reflex activity were always delayed In spite of the bulk of literature testifying to the value of aneurine in the treatment of peripheral neuritis a large amount of unpublished evidence has accumulated in the last ten years suggesting that it is of little value As Spillane [140] points out the acceptance of liver and insulin therapy was not long delayed and if aneurine were of value in the treatment of polyneuritis irrespective of its origin we would not be ten to fifteen years debating the problem

Shortly after aneurine became available commercially in 1935 Vorhaus Williams and Waterman [271] studied the
aneurine daily to 100 patients with neur
metabolic neuritis 20 with neuritis of
neuritis 11 associated with pernicious anaemia and 3 with pregnancy The patients were observed for periods varying from three to fourteen months 48 improved 44 were symptom free and 8 failed to benefit Russell [434] and Sciclounoff and Broccard [435] treated a number of mixed cases of neuritis with apparent benefit Russell used doses of 0 8 to 1 6 mg which would be present in a normal diet Vorhaus [436] treated 520 mixed cases of polyneuritis with 3 to 10 mg aneurine daily for an average period of nine weeks and concluded that treatment produced remission of symptoms in sixty one per cent and partial improvement in thirty six per cent Fifty per cent however had recurrences at the end of a year and ninety per cent at the end of three years Re administration of aneurine was effective in recurrent cases The weak link in these reports is the absence of controlled observations and failure to consider the natural history of the disease which often responds to rest and home treatment

Kalaja [755] and Goth [864] treated a number of cases of neuritis of varying aetiology with aneurine and concluded that relief followed only in those cases associated with a deficiency of aneurine

Infective Neuritis. Herpes Zoster. Unsupported claims have been made that aneurine relieves the pain of herpes zoster [338, 440, 633, 716]. One observer [633] naively observed that aneurine was only effective when used with other remedies, and another [439] only obtained relief in two cases out of sixteen.

Poliomyelitis. It has been claimed that aneurine deficiency is a predisposing cause of poliomyelitis [714]; however, the excretion of aneurine in children suffering from the disease is within normal limits [867]. In the rat a deficiency of aneurine *increases* the resistance of the animal to the Lansing strain of poliomyelitis virus [904]. Several workers have claimed that the administration of aneurine has a beneficial effect on the clinical course of poliomyelitis [444, 445, 745, 865]. Only small groups of patients were treated and no account was taken of the variability of the course of this disease. It is well known that some cases recover with virtually no paralysis, others remain badly paralysed, and in bulbar cases a fatal outcome is common. The assessment of any remedy in the treatment of this disease is not easy.

Diphtheritic Paralysis and Polyneuritis. It has been claimed that the duration of paralysis occurring in diphtheria patients can be shortened by the administration of aneurine [447–451]. Dieckhoff [453], Donovan and Bannister [707], Boe [219] and Wassman [373], in controlled observations, found no evidence that aneurine had a prophylactic or therapeutic effect on diphtheritic paralysis or on the cardiovascular complications of diphtheria. Reinhard and Schwartzer [451] recorded low excretions of aneurine in diphtheria patients (which may have been the result of the pyrexia) but they failed to observe any appreciable improvement in patients receiving the vitamin. Experiments on guinea-pigs injected with diphtheria toxin have shown that aneurine has no prophylactic or curative effect on post diphtheritic paralysis [373, 453]. Remarkable improvement from day to day may be observed in patients with post-diphtheritic neuritis in the absence of any treatment whatever, and rapid improvement may occur both early and late in the course of the disease.

Neuritis in Leprosy and Tuberculosis. Leprosy is frequently complicated by neurological lesions such as localized anæsthetic patches, neuritic tenderness and swelling of peripheral nerves. It is stated that pain, tenderness and swelling of affected nerves are diminished by administration of aneurine [508–511]. According to Aring and Spies [433] neuritis in tuberculous patients is relieved by injections of 50 mg. of aneurine.

Nutritional or Metabolic Neuritis. A number of cases of neuritis appear to be directly related to defective nutrition and may be termed nutritional or metabolic neuritis. The better-known conditions of this type in which neuritis is encountered are pernicious anæmia, alcoholism, pregnancy, diabetes, pellagra, sprue, nutritional neuropathy, malignant disease and gastrogenous neuritis.

Pernicious Anæmia. Russell [434] and Aring and Spies [433] state that the neuritic symptoms that occur in pernicious anæmia are relieved by aneurine. The latter used doses of 50 mg. twice daily. Defective absorption of aneurine may occur in pernicious anæmia patients owing to achlorhydria. There is no evidence that aneurine has any effect on the cord symptoms of pernicious anæmia [868], which are both prevented and relieved by vitamin B_{12} (p. 159).

Pellagra and Nutritional Neuropathy. Spies and his associates [374, 376, 461] reported relief of the symptoms of polyneuritis in pellagrins with aneurine, although they frequently observed deterioration of the mental state in the absence of other therapy. Lewy and his colleagues [378] observed that aneurine relieves the paræsthesiæ, numbness, pain in the calves, weakness and ataxia in pellagrins, while nicotinic acid had no such effect. They also made the interesting observation that large doses of aneurine precipitate the signs of riboflavine deficiency in pellagrins. It is generally considered that

aneurine alone affords little relief in nutritional neuropathy such as seen during the last war in malnourished prisoners of war natives and internees [146 384, 404 419 420] This condition is undoubtedly a multiple deficiency disease

Alcoholic Neuritis Until comparatively recently alcoholic neuritis was considered to result from the direct toxic effect of alcohol In 1933 Wechsler [403] treated three cases of alcoholic neuritis by dietetic means and considered that the neuritis was due to a deficiency of vitamin B_1 or B_2 At the same time Blankenhorn and Spies [405] observed that treatment of fifty alcoholics with neuritis by means of ets supplemented with yeast or liver extract he neuritis They also noted that there was if alcoholics were allowed as much alcohol as they could consume provided they received an adequate diet with yeast or liver injections Similar observations were made by Strauss [462] The chronic alcoholic drinks liquor instead of eating and the gastritis and anorexia produced restrict still further the intake of food while there is diminished absorption of what food is consumed Jolliffe Colbert and Joffe [266] calculated the aneurine requirements of forty two alcoholics and claimed that there was a correlation between the degree of aneurine deficiency and the presence of polyneuritis and that improvement varied with the intake of aneurine administered for treatment In 1938 Goodhart and Jolliffe [268] compared a group of alcoholics given treatment with intravenous injections of 10 mg of aneurine daily with another control group receiving what was considered to be an adequate diet The aneurine group recovered more rapidly and completely than the control group Other workers at this time recorded similar successes treating alcoholics with aneurine [463 468] The conclusions of these workers were criticized by Meiklejohn [698] who pointed out that even if the diets of alcoholics are lacking in aneurine it does not prove a causal relationship between aneurine deficiency and polyneuritis A diet lacking in aneurine is also likely to be lacking in other factors and the administration of aneurine may result in an increased food intake and hence these other factors

Subsequent experience has not confirmed these earlier enthusiastic reports which were generally based on uncontrolled observations on small groups Brown [562] studied 236 alcoholics over a period of eighteen years at the Boston City Hospital The average time spent by 118 of the patients on orthodox diet and treatment was compared with the same number given aneurine liver and the vitamin B complex The vitamin treated group were not discharged from hospital any sooner than the control group ability to

severity or duration of a case of alcoholic polyneuritis mitigated by them (i e *aneurine or the vitamin B complex*)

According to Jolliffe [406] aneurine is necessary for the metabolism of alcohol and an alcoholic therefore has an increased need for aneurine It has been postulated that the acetaldehyde formed by the metabolic oxidation of alcohol condenses with pyruvic acid with cocarboxylase as an enzyme a reaction that can occur *in vivo* [410 411] This suggests the necessity of aneurine for the metabolism of alcohol Lowry and his colleagues [412] however found that the polyneuropathy in aneurine deficient rats is delayed by alcohol and Westerfeld and Doisy [416] showed that the isocaloric substitution of alcohol for dietary fat or carbohydrate delays the onset of acute aneurine deficiency in pigeons If these observations are applicable to man—there is of course no evidence that they are—they suggest that the consumption of alcohol may actually reduce the requirements of aneurine

Pregnancy Neuritis Neuritis may occur in pregnancy According to

Vilter Morgan and Spies [270] this is a manifestation of beriberi in pregnancy and does not develop if the diet is adequate in aneurine. The condition, which frequently follows hyperemesis gravidarum, is characterized by generalized weakness and malaise, followed by tingling, burning of the feet and other paraesthesiae. The neuritis affects mainly the legs, is of a stocking distribution and is mainly symmetrical. There is tenderness of the calf muscles, hyperaesthesia on stroking the skin, hyperactive reflexes early in the condition and diminished or absent reflexes later. Foot drop may develop later and atrophy of the muscles and skin and vasomotor disturbances, such as sweating and cyanotic mottling, may occur. Degeneration of the myelin sheath, swelling of the cells, loss of Nissl substance and eccentricity of the cell nuclei have been described in the peripheral nerves [270, 869]. Undoubtedly pregnancy and lactation may predispose to nutritional deficiency in poorly nourished women. Such cases are rarely seen in this country but are more common in the East and in certain parts of America [565]. Low blood and urine levels of aneurine have been recorded in pregnancy [614, 566], this may not be a sign of aneurine deficiency, but of more efficient utilization. The cause of the neuritis in pregnancy is obscure—it frequently clears up spontaneously and after parturition—and, in spite of the claims of several observers that it is relieved by the administration of aneurine [264, 265, 501, 558, 568, 614, 870] it is not certain that lack of this vitamin is the cause. Neuweiler [421] obtained variable results. Other diagnoses have been suggested such as brachial neuritis, lumbodorsal root pains, acroparaesthesiae and first dorsal rib pressure syndromes [265, 501]. True generalized severe polyneuritis is rare in pregnancy [869]. It occurs between the twelfth and twenty fourth week of pregnancy and is preceded by very severe intractable vomiting and by emaciation. This may result from a condition of nutritional deficiency, but the factors responsible are unknown.

Gastrogenous Neuritis. Gastrogenous neuritis, associated with gastro intestinal disease, has been described (pp 225, 230). Ungley [260], Scott [261], Laurent and Sinclair [422] and others record cases of neuritis following gastric ulcer, pyloric stenosis, cardiospasm, dysphagia and neoplasms of the gastro intestinal tract. Undoubtedly chronic lesions of the gastro intestinal tract interfere with the digestion and absorption of food. Polyneuritis associated with dysentery and typhoid is described in text books of medicine but whether this is due to defective absorption of food or to a direct action of a toxin on the nerves is not known. The apparent relief of the symptoms of gastrogenous neuritis with aneurine is not conclusive, although it is rational to administer vitamins parenterally in bad cases, to patients with chronic gastro intestinal conditions and to patients on restricted diets. Spillane [146] points out that bedridden patients whether suffering from nutritional deficiency or not, may develop neuritis and palsies of the limbs from pressure.

Pressure Neuritis. It has been claimed that pressure neuritis due to neoplastic growths has been relieved by the administration of aneurine [191, 479]. The evidence however, is not very convincing.

Diabetic Neuritis. That neuritis may occur in diabetics is well known. Jolliffe and his colleagues [703] have estimated that it occurs in two per cent of diabetics and claim that the administration of 10 mg of aneurine a day relieves the condition in eighty per cent of cases. Others have reported that aneurine has a beneficial effect [435, 436, 446]. Needles [871] and Rundles [423] however, were unable to observe any benefit, either from administering aneurine alone or with other B vitamins. There is no evidence that diabetic neuritis is associated with aneurine deficiency.

Toxic Neuritis. Neuritis can be caused by chemical toxins, but there is no conclusive evidence that it is the result of aneurine deficiency. Sexton and Gowdey [424] have pointed out the close similarity between arsenical encephalopathy and aneurine deficiency.

	Arsenical Encephalopathy		Aneurine Deficiency
1	Muscular weakness particularly extremities loss of muscular control pain	1	Muscular weakness vague pains lack of energy
2	Paræsthesiæ in feet and hands	2	Paræsthesiæ in feet and hands
3	Numbness sensory disturbances	3	Numbness in lower extremities loss of vibration sense muscle tenderness
4	Absence of patellar and Achilles reflexes	4	Absence of patellar and Achilles reflexes
5	Constipation	5	Constipation
6	Staggering gait	6	Staggering gait
7	Regional anæsthesia and areas of hyperalgesia	7	Areas of cutaneous anæsthesia and areas of hyperalgesia
8	Upward spread of paralysis	8	Gradual paralysis spastic ataxia
9	Mental depression psychosis confusion disorientation	9	Psychosis disorientation
10	Dilatation of capillaries hæmorrhages into the cord and cerebral cortex cerebral œdema and petechial hæmorrhages	10	Hæmorrhagic areas in the brain capillary dilatation
11	Destruction of ganglion cells and anterior horn lymphocytes	11	Disintegration of ganglion cells
12	Focal vascular lesions which permit escape of blood into perivascular tissues	12	Vascular degeneration of the cells of Schwann
13	Zones of predilection corpus callosum optic thalami caudate nuclei external capsule and brain stem	13	Lesions occur in periventricular grey matter around third ventricle mamillary bodies periaqueductal region corpora quadrigemina

Sexton and Gowdey found that oxophenarsine an arsenical used in the treatment of syphilis causes a significant derangement in carbohydrate metabolism as evidenced by a fall in the lactate pyruvate ratio (p 242) and a rise in the blood pyruvic acid The raised pyruvic acid suggests that carbohydrate metabolism is arrested at the pyruvic acid level at which aneurine acts They suggest that BAL (dimercaprol) and aneurine should be used in the treatment of arsenical encephalopathy Hughes [425] also stresses the resemblance to chronic arsenical poisoning and he also suggests that lead and thallium act as enzyme poisons and produce their toxic effects through a biochemical lesion in the metabolism of carbohydrate It is known that some arsenicals e g Lewisite interfere with carbohydrate metabolism by attacking the sulphur groupings of the protein to which aneurine is linked as part of an enzyme system [426]

Claims have been made that aneurine relieves the neuritis that may occur in poisoning with such agents as tobacco [492 493] arsenic [491 492] lead [483 484] mercury [494] thallium [485 486] carbon disulphide [487] and sulphonamides such as sulphacetamide [490] Most of the authors quoted mention one or two cases only without controlled observations Aneurine has been found to be ineffective in the treatment of polyneuritis due to orthotricresyl phosphate poisoning (Jamaica ginger paralysis) [433 428]

Localized Neuritis It has been stated that localized neuritis—such as sciatica sacro iliac intercostal ulnar crural and shoulder girdle neuritis—is relieved by the administration of aneurine [440 496 498 502 505] These claims have never been confirmed and were not controlled in any way It is well known that such conditions often improve without any specific treatment

Affections of the Cranial Nerves Relief in some cases described as

dramatic [524], has been stated to occur when patients suffering from trigeminal neuralgia are treated with aneurine [523, 524, 528]. Borsook, Kremers and Wiggins [528], who treated fifty-eight patients, state that it may be necessary to inject 10 mg. of aneurine daily for as long as six months to obtain maximum benefit. They claimed "marked improvement" in sixty-four per cent. of the cases treated. Rose and Jacobson [706] however, concluded that aneurine had no beneficial effect. As in many nervous conditions, the pain of trigeminal neuralgia shows so many spontaneous remissions that very carefully controlled observations are necessary in evaluating a new treatment.

According to Selfridge [530] deafness resulting from lesions of the eighth nerve is improved by injections of aneurine and nicotinic acid. Seven cases were treated by Vesey [531], who found that the results varied but was inclined to think that aneurine was of some value. Aneurine has also been used in the treatment of neuritis of the eighth cranial nerve [573] and tinnitus [658]. Childrey [681] used aneurine for the treatment of non-specific deafness but found that very few cases received any benefit, which he confirmed by audiometer tests. He also treated several cases of tinnitus with aneurine and obtained relief in only one case. Using audiometer tests, Shambaugh and Jennes [875] were also unable to confirm the value of aneurine in the treatment of nerve deafness and tinnitus aurium. They state that many of the claims are based on the subjective evidence of patients and are insufficiently controlled. The evaluation of therapy for the improvement of hearing must be based on repeated audiometric tests, and to be significant there must be a sustained improvement of at least 10 decibels above the pre-treatment level.

Harris and Moore [544] state that daily doses of 20 mg. of aneurine and 250 mg. nicotinic acid produced remarkable improvement in patients with Ménière's syndrome. Of twenty cases, seventeen became entirely free from vertigo and the remaining three showed considerable improvement. Treatment was carried out for two to three months before maximum benefit was obtained. Atkinson [329] also described the treatment of nineteen cases of Ménière's syndrome by similar means.

Claims have also been made that injections of aneurine are of benefit in the treatment of facial paralysis [529] and optic neuritis [532, 533]. They have not been confirmed.

Diseases of the Spinal Cord. *Disseminated Sclerosis.* Moore [535] claimed that in five advanced cases of this disease treated with aneurine and nicotinic acid there was considerable subjective and objective improvement, which was not maintained when treatment was stopped. Stern [513] also reported benefit after the intraspinal injection of large doses of aneurine. Roch and Sciclounoff [440] treated seven patients with aneurine, four of whom were stated to show definite improvement. These investigators, however, were careful to state that "this may perhaps have been due to a natural remission in the disease and to hospitalization rather than to our interventions." Other workers have failed to report any benefit [503, 524, 683]. Masek [536] treated a number of cases of disease of the central nervous system, including disseminated sclerosis, with aneurine but did not observe that it had any beneficial effects.

Tabes. Wintrobe and his co-workers [881] produced spinal cord lesions in pigs similar to the lesions of tabes dorsalis in man by feeding them diets deficient in the vitamin B complex. This prompted the trial of aneurine in the treatment of tabes. Metildi [537] and Reese and Hodgson [538] claim that the administration of aneurine, liver extract and yeast produces relief of pain, diminished ataxia and an increase in visual fields and sensory power. Aring and Spies [433] also report relief after administering large doses of aneurine (100 mg.) during tabetic crises. Stone [639] treated sixty-three tabetic patients with intraspinal injections of aneurine and also vitamin B

complex preparations wheat germ oil arsenic and bismuth Some patients received fever therapy as well In seventeen out of twenty three patients with advanced tabes improvement occurred in gait bladder symptoms visual disturbances and lightning pains The greatest improvement was shown in those receiving fever therapy Kesert and Grossman [480] also report relief of lightning pains and gastric crises after injecting 50 to 100 mg of aneurine intraspinally Lack of controls reliance on subjective symptoms and the multiplicity of treatments make such work difficult to evaluate The anti syphilitic therapy alone may have produced some benefit Cochems and Kemp [882] treated a large group of tabetics with injections of aneurine for eight months only nineteen per cent obtained relief and definite proof of the value of aneurine in the treatment of tabes was lacking These observations were made before the treatment of neurosyphilis with penicillin

Pernicious Anæmia (Cord Symptoms) It has been claimed that the neurological complications of pernicious anæmia are relieved by injections of aneurine [716] although Aring [868] was unable to verify this It is now known that the cord lesions of pernicious anæmia can be prevented by and cured with vitamin B_{12}, which appears to be the true extrinsic factor

Other Diseases of the Nervous System Several investigators have reported that aneurine produces some improvement in conditions such as Parkinsonism progressive muscular atrophy pseudohypertrophic muscular dystrophy Friedrich s ataxia amyotrophic lateral sclerosis and Sydenham s chorea [540 685] These reports have not been confirmed [572 607]

Relief of Pain Some investigators have reported that aneurine has an analgesic action Stern [513] states that the intraspinal injection of 10 to 100 mg of aneurine produces relief of the intractable pain occurring in such conditions as incurable cancer disseminated sclerosis thromboangiitis obliterans tabes Paget s disease and osteoporosis of the spine [513] Aring and Spies [433] however were unable to confirm this reputed analgesic action in patients suffering from severe pain of nervous origin (carcinoma cord tumour trigeminal neuralgia) even when it was given in large doses Krieg [521] and Ochsner and Smith [522] claim that parenteral or oral administration of aneurine is effective in relieving the pain of varicose ulcers and Shosberg [515] has reported relief of pain in cases of painful amputation stumps There is some evidence that aneurine relieves pain in nutritional neuropathy [146] although why it should do so in the above cases is obscure Pharmacologically it is a vasodilator but it is not known whether it is so in the doses in which it is used clinically The vasodilator action of aneurine may explain its action in relieving the pain of migraine which is reported by Bandler [627] and Palmer [872] Palmer states that an injection of 60 to 120 mg of aneurine intramuscularly can terminate an attack of severe migraine unaffected by ergotamine tartrate

Aneurine has been used in dentistry to relieve the pain of dry socket after dental extractions [873 874]

Summary In spite of the early enthusiastic reports on the use of aneurine in the treatment of conditions of the nervous system these have not been confirmed There is very little evidence of its value in the treatment of any neurological condition unless this is definitely associated with a deficiency of aneurine either of intake or conditioned (p 223)

Neuropsychiatric Disorders It has been claimed that the simultaneous injection of 2 to 10 mg of aneurine minimizes the occurrence of convulsions

It has been assumed without any evidence that since aneurine is essential for the metabolism of carbohydrate and since only the latter is used as a source of energy by the nerve cell that aneurine administered therapeutically can effect the higher cerebral processes This has led to its use to

cases the deficiency states have been multiple and not pure aneurine deficiency and vitamin B therapy has often meant the administration of concentrates containing not only aneurine but also other vitamins of the B complex It is therefore difficult to assign an exact role to the effects of aneurine in these studies Where aneurine deficiency can be incriminated as the cause of anorexia or is associated with ulcerative colitis vomiting diarrhoea gastro intestinal diets hepatic cirrhosis or impaired absorption then the vitamin should be given in adequate amounts preferably by parenteral injection if absorption by mouth is debatable There is no evidence that aneurine has any effect on the condition itself [739] although Cheney [598] describes the dramatic response of thirty two cases of chronic diarrhoea and mucous colitis to treatment with aneurine

Field Robinson and Melnick [570] have found that patients receiving intensive alkali therapy for peptic ulcer and those with achlorhydria have subnormal excretions of aneurine *In vitro* experiments showed that as much as fifty six per cent of aneurine is destroyed when incubated with bile or pancreatic juice in the absence of acid gastric juice Patients suffering from peptic ulcer or achlorhydria may therefore develop an aneurine deficiency unless they take in more of the vitamin than will protect a normal individual

Many claims have been made that aneurine is of value in the treatment of gastro intestinal hypotonia It has certainly been observed that the administration of foodstuffs rich in aneurine is helpful in overcoming atonic constipation but the effect of the bran and fibre in these foodstuffs cannot be overlooked The laxative action of yeast is also well known There are many reports of success in the treatment of constipation by means of pure aneurine or a concentrate but the difficulty of evaluating these reports is considerable especially when defaecation with some individuals is almost a conditioned reflex [182]

Careful X ray studies have been made by Wood Splatt and Maxwell [642] on the effect of aneurine on gastric secretion and motility in man They state that in doses of 3 to 10 mg intramuscularly it has no effect on gastric secretion although it hastens the emptying time in those persons whose gastric emptying time is habitually much longer than normal It does not influence the rate of evacuation of the stomach of those whose emptying time is normal or rapid Controlled studies on the supposed laxative action of aneurine have also been made by Loewe and Knox [643] in the rhesus monkey the only animal suitable for measuring the effectiveness of cathartic drugs They found that in doses of 1 to 100 mg per kilo orally for periods of two to seventeen days it did not increase significantly the laxative effect of phenolphthalein While a deficiency of aneurine may lead to an atonic condition of the bowels there is little evidence that the vitamin has a laxative effect in adequately nourished persons It is possible that the clinical material for the observations recorded above was selected from poorly nourished hospital patients

So called gastro intestinal diets are traditionally over supplied with starches and sugars and with foods containing insufficient vitamins Functional disorders of the gastro intestinal tract are frequently related to insufficient supplies of the vitamin B complex [602] In such circumstances restriction to certain of the therapeutic diets will have an additive effect and may precipitate deficiency disease Where anorexia is associated with aneurine deficiency the administration of the vitamin restores appetite [605]

It is believed that an insufficient intake of aneurine may be responsible for some gastro intestinal symptoms in children and babies Wilkins [324] records striking improvement in some cases among children after giving small doses of aneurine Partial aneurine deficiency was also described by Clements [645] in at least eight per cent of 150 infants breast fed up to six months The symptoms attributed to aneurine deficiency were failure to gain weight at the normal rate constipation and vomiting The adminis

tration of aneurine to the child or to the mother if still nursing cured these symptoms It should be noted that in these cases a claim is made for treatment with aneurine only in the presence of a definite deficiency of the vitamin

given from 2 to 5 mg of aneurine and not less than 50 mg of ascorbic acid per day should be administered

Aneurine in Pregnancy Reference has already been made to the increased need of aneurine in pregnancy Some writers believe aneurine deficiency to be an ætiological factor in such disturbances of pregnancy as hyperemesis gravidarum pregnancy neuritis cardiovascular disorders and the toxæmias of pregnancy While aneurine deficiency may cause neuritis and cardiovascular disturbances which are amenable to treatment with the vitamin it is more likely that hyperemesis gravidarum and pregnancy toxemia cause aneurine deficiency the former by repeated vomiting and loss of gastric hydrochloric acid needed for the absorption of aneurine (p 226) the latter by hepatic dysfunction interfering with efficient utilization of aneurine (p 226) Wernicke s encephalopathy (p 231) which has been attributed to aneurine deficiency is a terminal phase in hyperemesis gravidarum [734 735] and Korsakoff s psychosis has also been reported [611]

Nixon [734] refers to the triad—œdema toxæmia and aneurine deficiency—frequently seen in pregnant women in Hong Kong The report of the University Clinic there shows an alarming increase in the number of cases of eclampsia and beriberi the more severe the eclampsia the higher the incidence of aneurine deficiency with concomitant increased mortality Forty five per cent of the cases of eclampsia were complicated by aneurine deficiency From a study of eight cases in this country Nixon showed that the excretion of aneurine was below that of normal controls and the aneurine content of the placentæ of eclamptic patients was also significantly low

function may be a contributory factor in the precipitation of pregnancy toxæmia These observations of Nixon have been confirmed by King and Ride [470] Neuweiler and Nyffenegger [596] state that there is a rise of blood bisulphite binding substances (p 242) in hyperemesis gravidarum

Most of the writers associating aneurine deficiency with the toxæmias of pregnancy diagnose the former from a low urinary excretion of aneurine The fallacy of this has been pointed out before (p 243) Rose and his co workers [560] failed to note any decrease in the toxæmia during the latter part of pregnancy in a controlled group of patients receiving 3 mg of aneurine and other members of the B complex daily Horwitz and Farley [566] concluded from blood

suffering from ane

severe hyperemes

have caused a co

a nutrition survey of over 500 pregnant women noted that eighty four per cent of a group showing excessive nausea and vomiting in early pregnancy had an aneurine intake of less than 2 mg daily It is difficult to say whether the low aneurine intake was the cause of the nausea and vomiting The intake of other essential nutrients was also probably low We would like to re emphasize the view that vitamin deficiencies are never limited to lack of a single vitamin

The use of aneurine in the treatment of toxæmia of pregnancy has been disappointing Yasunami [559] working in Japan states that he found

it effective in the treatment of the condition particularly in preventing convulsions in pre eclamptic patients This has not been confirmed by work in America and Europe Siddall [325] although he claims that the toxæmias of pregnancy are associated with a deficiency of aneurine, failed to observe any improvement in patients with pre eclampsia after giving them daily injections of 1 to 7 mg of aneurine for ten days Strauss [657] Browne [886] and Kapeller Adler and Cartwright [887] failed to observe any significant improvement in blood pressure œdema or albuminuria in patients with toxæmia of pregnancy treated with aneurine in doses from 9 to 25 mg daily Kapeller Adler and Cartwright [887] state that in severe cases aneurine actually intensified the signs and symptoms of pre eclamptic toxæmia in the patients studied They consider its use contra indicated in view of the fact that it inhibits histaminase an enzyme that normally hydrolyses histamine in the body Kapeller Adler believes that in normal pregnancy most of the histamine formed in the body is destroyed by histaminase but that in the toxæmias of pregnancy it escapes destruction

Wiedenbauer [609] Spitzer [610] Bernstein [571] Lund [568] and Willis [888] claim to have effectively treated hyperemesis gravidarum with aneurine In some cases other preparations such as ascorbic acid suprarenal cortex hormone and pyridoxine were used Spitzer used doses of 10 to 20 mg of aneurine Willis and others [888] gave doses of 25 to 50 mg daily parenterally up to a total dose of 800 mg The results were stated to be satisfactory but not as good as with pyridoxine Most of these observations were not controlled and their evaluation is, therefore difficult Hyperemesis causes aneurine deficiency (p 223) but why the administration of large doses of aneurine should cure the former is difficult to understand It may of course restore appetite and improve liver function by improved carbohydrate metabolism Success has been claimed for many forms of treatment of hyperemesis but it is probable that the success is due to the psychological effect of the treatment, particularly if this involves the use of the hypodermic needle

According to Bickel [442] cardiovascular disturbances due in certain cases at least to aneurine deficiency, may develop during pregnancy in women who were previously apparently normal He states that they are likely to appear in connection with pregnancy toxæmias Daily injections of 50 mg of aneurine were stated to produce a cure Stahler [614] describes similar cases treated with injections of 10 mg daily

Large doses of aneurine and the B vitamins are said to relieve heartburn in pregnancy [889] As aneurine has no spasmolytic action and has no effect on gastric secretion and motility its use for this purpose does not seem to have any rational basis

Aneurine in Metabolic Diseases Diabetes Certain workers claim to have improved the carbohydrate tolerance of diabetics by administering aneurine Thus Vorhaus and his co workers [271] state that they obtained improvement in diabetes treated with aneurine particularly in patients suffering from obesity lack of appetite and a diminished metabolic rate Sciclounoff [291] states that twelve of thirty five diabetics showed improvement for periods of several days up to some months after supplements of aneurine the glucose tolerance test curve was also lower after taking aneurine Dienst [616] stabilized a group of diabetics on insulin and administered a preparation containing aneurine and claimed that carbohydrate tolerance was so improved that the patients required 10 to 20 units of insulin a day less Hypoglycæmic reactions were said to be fewer A fall in blood sugar and diminished glycosuria after administering aneurine to diabetics has also been reported [617]

Others have not been able to reproduce these results Lawrence and Oakley [443] treated a large series of diabetics with aneurine but could not record any effect for better or worse, on the carbohydrate tolerance or insulin

requirement Kaufman [670] could not observe that aneurine had any effect on the blood sugar of diabetics Smith and Mason [555] kept two patients with severe diabetes on aneurine deficient diets and a third was given injections of glucose with and without the addition of aneurine the vitamin had no effect on the severity of the diabetes or on the insulin requirements of the patients Trasoff and Bordin [741] Robson [452] and Owens [684] could not observe any discernable effect on the severity of the disease after administering aneurine and the vitamin B complex to diabetics

The relatively mild degree of depression of carbohydrate tolerance seen in animals and human beings after long periods of aneurine deprivation appears to represent a disturbance of metabolism unrelated to that involved in diabetes Such "false diabetes can be corrected by administering aneurine true diabetes cannot Glucose tolerance is impaired in the the late stages of aneurine deficiency but it must be both prolonged and severe [334]

No reduction in the insulin requirements of diabetics receiving large amounts of aneurine has been observed [684], it is believed that aneurine deficiency may play a part in the causation of diabetic coma in which the most severe damage falls on the brain heart and kidneys the cells of which are very susceptible to lack of aneurine That disordered carbohydrate metabolism may occur in a diabetic coma is suggested by the finding by Markees and Meyer [460] of a raised blood pyruvate in diabetic acidosis These investigators reported that recovery from diabetic coma induced in rabbits experimentally with alloxan is hastened by treatment with cocarboxylase (aneurine pyrophosphate) and riboflavine They further state that a number of diabetics (unspecified) in coma have been treated with cocarboxylase with beneficial results the patients recovering more rapidly the alkali reserve rapidly increasing and the blood pyruvate falling [469] Boulin and his co workers [470] have made similar claims

Gilliland and Martin [473] have confirmed the raised blood pyruvate in diabetic acidosis in alloxan diabetic rabbits and in diabetic patients with acidosis but supplementing standard treatment for diabetic acidosis with cocarboxylase and riboflavine as recommended by Markees and Meyer [469] did not accelerate recovery as measured by clinical improvement a fall in the raised blood pyruvate and blood sugar and a rise in the alkali reserve

It has been assumed that the aneurine requirements may be increased in diabetics In theory the diminished oxidation of carbohydrate would be expected to decrease the requirement of aneurine This has been shown experimentally by Lowry and Hegsted [477] who showed that the aneurine requirement of the animal suffering from alloxan diabetes is less than that of normal controls The animals showed no increased tendency to develop signs of aneurine deficiency Caution must of course be exercised in the interpretation of such experimental findings as alloxan diabetes differs from human diabetes particularly with regard to the capacity to survive without the help of insulin

Hyperthyroidism There is a superficial resemblance between the symptomatology of hyperthyroidism and aneurine deficiency—anorexia diarrhœa cardiac enlargement tachycardia fatigue palpitations impaired muscular strength disturbed carbohydrate metabolism and neurasthenic symptoms For this reason the administration of aneurine and the vitamin B complex has been recommended in the treatment of hyperthyroidism The basic pathology of the two conditions is however quite different In one the raised metabolism throws a strain on the cardiovascular system in the other a deficiency of aneurine produces pathological changes in the heart muscle Williams [296] and Davis and Bauer [936] observed a low blood cocarboxylase and a raised blood pyruvate in thyrotoxic patients Williams found no correlation between these figures and the B M R

Cowgill [198] administered 60 micrograms of aneurine per 100 calories of

the estimated total daily metabolism which is increased in hyperthyroidism Means and his associates [620] state that the clinical course of hyperthyroidism is favourably influenced by administering aneurine although Jacobi and Pomp [621] could not confirm this controls treated with rest and diet doing just as well as those receiving additional aneurine and vitamin A Frazer and Ravdin [121] supplemented the routine pre operative preparation of twenty eight patients for thyroidectomy with 10 mg of aneurine hypodermically every other day and 10 grams of yeast daily and compared the results obtained with a control group of hyperthyroid patients not receiving aneurine The aneurine had no antithyrotoxic action nor had it any effect on the B M R or on the severity of the post operative crisis It was considered however to bring down the pulse rate increase weight and appetite and to diminish the time needed for pre operative preparation Williams [?96] prescribed brewer s yeast and 10 to 20 mg aneurine daily to all thyrotoxic patients under his care for four years, a distinct subjective improvement was recorded Williams considers that the aneurine requirements are increased in hyperthyroidism as aneurine is lost to the body in the sweat fæces and urine as a result of hyperhidrosis diarrhœa and diuresis

Gout Vorhaus and Kramer [624] have reported relief of pain in acute gout by administering aneurine Kuhnau [625] states that the blood nucleotides are raised in gout (5 to 10 mg per millilitre normal 2 to 4 mg) Birch and Mapson [626] have observed a similar rise in beriberi Kuhnau records that in patients with gout the intravenous injection of 10 to 20 mg of aneurine is followed by ... lness and a fall in the blood nucleotide ... lase removes uric acid from the ... outy subjects the formation of purines is so increased that the normal a ount of aneurine in circulation is insufficient for its removal Callahan and Ingham [575] treated nine cases of gout with 15 to 30 mg of aneurine daily They state that the period of disability was considerably reduced although at the same time they diminished the purine intake of the patients administered cincophen and treated them with medicated baths

Dermatology Several workers have published uncontrolled observations on the treatment of skin conditions with aneurine There is no rationale for its use and no evidence that it is effective in the treatment of any disease of the skin

Shock Govier and Greer [749] state that the average survival time of anæsthetized dogs in which hæmorrhagic shock has been induced is significantly greater in those animals treated with aneurine than in untreated animals The dose given was 1 to 2 mg per kilo followed by 0 5 mg every two hours The average survival time in the treated group was eight hours in the untreated three and a half The administration of aneurine lowered the level of keto acids lactic acid and sugar in the blood of the bled dogs This work was repeated with rabbits by Maycock [534] who was unable to confirm it Govier [641] has studied the relationship between hæmorrhagic shock and the plasma aneurine level He states that the resistance to shock is greater in animals with a raised plasma aneurine they withstand more bleeding—forty five per cent more than controls with a low plasma aneurine —before developing severe hypotension and they show a more rapid return to their normal blood pressure when hæmorrhage stops Govier and Grieg [652] have shown that in dogs subjected to shock from hæmorrhage and in animals suffering from anoxic anoxia dephosphorylation of cocarboxylase occurs If the bled dogs are given aneurine a resynthesis of cocarboxylase results ... shock be ... its benef ... cocarboxylase Alexander [915] has shown that the concentr ... and phosphorylated aneurine in the liver rises in prolonged hæmorrhagic

shock The non phosphorylated aneurine of muscle also shows an increase, occurring at the expense of cocarboxylase

It has been shown that in patients with severe injuries hæmorrhage infection and in those suffering from burns there are considerable alterations in aneurine metabolism as shown by a low urinary excretion [488 489] The metabolism of nicotinic acid riboflavine and ascorbic acid is also affected and it is suggested that 10 to 20 mg of aneurine be given to such patients for the prevention of shock Bergman and others [495] however were unable to show that the administration of aneurine or ascorbic acid had any beneficial effect in the treatment of shock due to scalding burns in mice

According to Grieg [499] destruction of three co enzymes may occur in

from shock or conditions likely to result in shock such as extensive burns trauma and surgical procedures Lund [889] recommends 10 to 20 mg each of aneurine and riboflavine 150 to 250 mg of nicotinic acid and 1 to 2 grams of ascorbic acid These vitamins alone do not of course satisfy the nutritional needs of the patient which include a high protein high carbohydrate diet supplemented by yeast liver or liver extract

Irradiation Sickness It is claimed that the administration of aneurine affords some relief from the symptoms of irradiation sickness which is characterized by nausea vomiting diarrhœa nervous symptoms and head ache and which may occur after exposure to therapeutic doses of X rays or the rays from radium Martin and Moursund [654] state that these symptoms are prevented by giving 6 mg of aneurine orally and a high carbohydrate diet for at least two days before exposure commences If vomiting occurs it is frequently relieved by an intramuscular injection of 6 mg Imler and Wammock [656] and Sponheimer [655] report that injections of 10 mg of aneurine daily give rapid and complete relief from the more severe symptoms of irradiation sickness in the majority of cases In severe cases the dose is increased from 15 to 30 mg daily by injection Results following parenteral therapy are said to be more effective than those following the oral route

with a combination of aneurine and nembutal Whitmore [336] from observations on 122 cases found that 6 to 9 mg of aneurine daily prevented symptoms of irradiation sickness in eighty per cent of the cases The dose was increased if sickness developed Another report from Bean Spies and Vilter [940] favours the administration of 50 mg of aneurine and 300 to 500 mg of nicotinic acid daily These workers state that the incidence of irradiation sickness is greater in patients consuming diets poor in the vitamin B complex Once irradiation sickness was established the administration of aneurine and nicotinic acid had little effect in relieving it although it was found that if given in the doses stated before exposure the onset of the sickness was largely prevented Bean Spies and Vilter suggest that the basic disorder in irradiation sickness is a disturbance in the respiratory enzyme systems of which aneurine and nicotinic acid are components

Aneurine and the Sulphonamides Fleisch and De Preux [894] state that albuminuria and hæmaturia due to sulphonamides such as sulphapyridine sulphathiazole and Irgamid (N dimethylacroylsulphanilamide) can be diminished by administering large doses of aneurine aneurine has no effect however on the acute toxity or lethal dose of the drugs in animals Higgins [900] noted that the toxic effects of the sulphones—hyperæsthesiæ loss of weight anorexia anæmia and lassitude—can be largely prevented in rats if they are given six times the normal allowance of aneurine riboflavine and

pyridoxine. Yeast, however, is ineffective, presumably because it cannot supply such large quantities of the B vitamins.

According to Kinnunen [500] aneurine plays a fundamental role in the acetylation of the sulphonamides. It increases the acetylation of sulphonamides in the rabbit, possibly by increasing the formation of the acetylating component, believed to be either ketene or acetyl phosphate, from the products of carbohydrate breakdown (pyruvic acid). Kinnunen has shown that sulphapyridine interferes with the formation of citrate in the carbohydrate cycle (p. 195). He believes that the neurotoxic effects of the sulphonamides are due to their acetylation, which interferes with the formation of acetylcholine. The reputed protective effect of aneurine (p. 259) is due to its catalysing the oxidation of pyruvate, which makes more of the acetylating component available.

The effects of the sulphonamides producing a partial aneurine deficiency by interfering with its intestinal synthesis (p. 204) has probably been greatly exaggerated. The administration of phthalylsulphathiazole to normal subjects makes very little difference to the excretion of aneurine in the urine or in the faeces [127]. In the rat also the administration of sulphonamides does not influence the urinary excretion of aneurine, suggesting that normally little of the aneurine synthesized in the gut by bacteria is absorbed.

According to Slater [619, 623] sulphadiazine and sulphamerazine have an aneurine-sparing effect in man and the rat; sulphanilamide, sulphathiazole, succinylsulphathiazole and sulphapyridine have no such effect. The two former sulphonamides may act on the metabolic rate, as they are the only sulphonamides which produce thyroid enlargement.

Uterine Cancer. Deficiency of vitamin B has been postulated as a factor in the ætiology of uterine carcinoma; this has led to the suggestion that the B vitamins might be used prophylactically against the development of cancer. Ayre and Bauld [541] state that women with carcinoma of the cervix uteri show signs of aneurine deficiency and hyperœstrinism as evidenced by vaginal smears. They then postulate that in the presence of chronic vitamin B deficiency the liver does not inactivate endogenous œstrogen, excess of which is localized in the cervix, which is often the seat of chronic cervicitis. Œstrogen then produces metaplastic and eventually carcinomatous changes in the epithelial cells of the cervix. These views are based on the work of Biskind [512], Segaloff [542, 543] and Singher and his co-workers [546], who found that in the aneurine and riboflavine deficient rat the liver loses its ability to inactivate œstrogen. They are also supported by the observation that patients with severe liver damage excrete greater amounts of endogenous œstrogen and excrete a higher percentage of administered œstrogen than normal [547]. More recent investigations, however, have shown that there is no significant difference between the level of aneurine nutrition in women suffering from carcinoma of the cervix and normal controls [549], and that the failure of the liver to inactivate œstrogen in vitamin B deficient rats is due not to vitamin B deficiency *per se*, but to inanition. Greene and Peckham [550] have critically reviewed the literature on the subject.

Resistance to Fatigue. There are several reports on the beneficial effects of giving aneurine either alone or with other vitamin mixtures to increase the capacity and resistance to fatigue of athletes, soldiers and men engaged in heavy work [337, 660, 700]. These observations, which were largely uncontrolled, have not been confirmed. Many critically controlled studies on the subject have since been made, and the conclusion drawn by four separate groups of investigators is that the administration of aneurine or members of the vitamin B complex to adequately nourished persons has no influence on work output, endurance in dynamic work, recovery of working capacity, or recovery from fatigue [338, 340, 796, 797]. In one group investigated there was no change in the heart rate, oxygen consumption, respiratory

quotient, urinary excretion of nitrogen and ketone bodies, and blood lactate, nitrogen glucose and B B S [797] The Council on Foods and Nutrition in America [341] has pointed out the waste of material and money in the indiscriminate administration of vitamin mixtures to workers in industry with a view to increasing output and diminishing fatigue As the Council points out if the workers are adequately nourished additional vitamins serve no useful purpose , if they are not adequately nourished they need more or better food and not vitamins out of a bottle On the other hand, it has been reported that on diets deficient in aneurine and the vitamin B complex there is a decreased work output in trained subjects, loss of ambition and efficiency, and poor recuperation [584, 697, 836, 890] This subject is dealt with in further detail on p 239

These observations are in keeping with those on rats Aneurine in large doses has no effect on the work performance of rats receiving adequate supplies in their diet [912] There is some evidence, however, that the vitamin may exert a pharmacodynamic effect on isolated perfused muscle The total work output of the gastrocnemius muscle of the frog is significantly increased by perfusion with fluids containing 0 01 milli equivalents of aneurine and calcium pantothenate per litre [943] This concentration of aneurine is actually many times greater than that occurring in skeletal muscle and the effects may well be due to vasomotor changes Lissak and his co workers [525] were unable to confirm this effect of aneurine on isolated muscle

Aneurine deficiency is not a significant factor in producing fatigue and other symptoms (effort intolerance, breathlessness palpitation præcordial pain and subjective feelings of fatigue) in patients with the effort syn drome [937]

Morphine Addiction Fitzhugh [666] observed that the irritability of rats addicted to increasing doses of morphine was reduced by aneurine The vitamin also prevented the increase in irritability that follows morphine withdrawal Himmelsbach [137] has been unable to confirm this clinically in the case of morphine addicts

Other Clinical Uses of Aneurine Aneurine has been used in the treatment of pink disease although the earlier enthusiastic reports have not been confirmed [516–519] It is a disease with remissions and exacerbations and eventual recovery or death from intercurrent infection such as broncho pneumonia Evaluation of a remedy in small numbers of cases is therefore difficult

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CHAPTER IV

RIBOFLAVINE

HISTORY

In 1932 Warburg and Christian [1] described a new 'yellow enzyme' which they obtained from aqueous extracts of bottom yeasts. They claimed that it played an important role in respiration by forming part of an oxidation reduction system acting as a carrier of molecular oxygen to an oxidizable substrate. Warburg and Christian [2] later separated this 'yellow enzyme' into a protein component and a pigment component and noted that neither alone was catalytically active. In 1933 reports appeared from three independent research groups suggesting a relationship if not identity between vitamin B_2 or G, a heat labile factor of the vitamin B complex, and the water soluble yellow green fluorescent pigments found in many animal and plant products such as yeast, liver and kidney. Kuhn [4, 5] and his associates in an attempt to isolate 'vitamin B_2' from eggs obtained a water soluble yellow green fluorescent pigment which like 'vitamin B_2' had growth promoting properties. They observed the similarity in distribution of the two substances and called attention to their probable relationship to the yellow enzyme of Warburg. Since pigments with apparent different vitamin activities were obtained from various sources they were looked upon as different members of a chemical group to which the term 'flavin' was applied. The various flavins were called lactoflavin, hepatoflavin, ovoflavin, etc., according to their origin. Subsequent research has shown that they are all identical. At the same time Booher [6] in America showed that the growth promoting activity of whey, which is a function of its 'vitamin B_2' content, runs parallel to the amount of yellow pigment present.

Subsequent studies confirmed the identity of vitamin B_2 with the yellow pigment. After the elucidation of its structure and synthesis it was decided in 1937 by the Council on Pharmacy and Chemistry of the American Medical Association to call it riboflavin and to abandon the terms vitamin G and B_2.

CHEMISTRY OF RIBOFLAVINE

Riboflavine was isolated from many natural sources by Kuhn [5] and his collaborators and their work has been confirmed by a number of other investigators. Among the sources were egg yolk and white, milk, liver, kidney, urine, grasses, fish retina, barley malt and yeast.

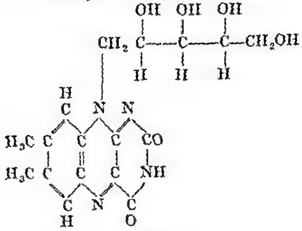
Riboflavine crystallizes in yellowish brown needles (Fig. 84). The needles have no sharp melting point but darken at 240° C and melt at 275°–282° C with decomposition. Although stated to be water soluble its solubility is very slight, being only 12 mg per 100 ml at 27.5° C. It is quite insoluble in the fat solvents. It is very soluble in alkali solutions. The solubility in water is increased by adding urea or by the formation of a complex with boron by means of which an 0.3 per cent solution may be obtained. The solubility is also increased by the presence of tryptophane or nicotinamide [317]. Riboflavine is stable in strongly acid solution but is unstable in the presence of alkali or when exposed to light or irradiation with ultra violet light, which cause irreversible decomposition. The vitamin is therefore stored in tubes covered with black paper or in amber coloured ampoules. When exposed to daylight in neutral solution the ribose chain is split off to form lumichrome, which shows a greenish yellow fluorescence but is devoid

of vitamin activity. T
atoms of the ribose t
and like lumichrome has no vitamin activity. Riboflavine is relatively highly thermostable (e g only slight destruction at 120° C for six hours) and uninfluenced by atmospheric oxygen in the dry state Solutions of riboflavine exhibit a strong yellow-green fluorescence which reaches a maximum between *p*H 6 0 to 7 0 In milk at least ninety per cent of the riboflavine appears to be in the free form In most other sources, such as yeast, liver, and plants it occurs conjugated with other compounds of high molecular weight

Following the isolation of riboflavine in crystalline form, studies by Warburg and Christian [2] and by Kuhn and Rudy [9, 10] in 1934, led to the

Fig 84 Crystals of Riboflavine

elucidation of its constitution. The synthesis of riboflavine, which was essential for the final proof of its chemical constitution, was effected in 1935 by Kuhn [11] and his co workers and by Karrer [12] and his collaborators D Riboflavine is 6 7 dimethyl-9 *d*-ribityl isoalloxazine.



D Riboflavine

The fact that riboflavine exhibits a blue green fluorescence in ultra violet light serves as a basis for determining it by fluorometric methods [17–22,

145 148 320 321] The vitamin can be separated from biological fluids or foodstuffs by adsorption on a cation exchange resin and eluted with pyridine [319] It can be detected in biological material by fluorescence micro spectroscopy [7] It is also estimated by converting it into lumiflavin by exposure to light in alkaline solution and then determining the lumiflavin colorimetrically Biological methods have been employed [14 15] A micro biological method has been developed which depends on the observation that the growth of a specific strain of *Lactobacillus casei* or *Lactobacillus helveticus* and the resulting production of acid is proportional to the amount of riboflavine in the medium [132 133 149 152 323] The physico chemical methods for estimating riboflavine are reviewed by Hoffman [13] and Skiller [321] and microchemical methods by Bessey [322]

UNITS OF RIBOFLAVINE

The Bourquin Sherman [16] method of estimation originally described as a method of assay for vitamin B_2 or G in the old nomenclature has been shown to be only an approximate measure of riboflavine The method is not specific since it is based on growth responses and many other factors besides riboflavine are essential for growth The Bourquin Sherman unit was defined as the amount of vitamin B_2 which when fed daily to a standard rat during eight weeks will cause an average gain of 3 grams per week in addition to the average gain of the control test animals fed on a vitamin B_2 free diet One Bourquin Sherman unit has been variously reported as being equivalent to from 2 to 7 micrograms of pure riboflavine The most recent figure is 2 19 micrograms [29] There is no international unit

Now that physico chemical methods are available for the estimation of the vitamin its concentration in a foodstuff or preparation can be expressed in milligrams or micrograms per given volume or weight of material

DISTRIBUTION OF RIBOFLAVINE IN FOODS

Riboflavine is widely distributed in plants which synthesize it and in animal tissues Among the best sources are yeast milk white of egg fish roe kidney liver heart and growing lea
sources are fish meat and poultry muscle
contain it are not particularly rich sources
during germination

In green vegetables the leafy portions and growing parts contain most riboflavine as the leaves get older and dry the riboflavine content diminishes It has been shown that the milk from cows fed on fresh young grass contains more riboflavine than those fed on dried or root crops which accounts for the fact that the riboflavine content of milk is highest in summer Milk eggs and leafy green vegetables are the chief sources of riboflavine in the average dietary The riboflavine content of human milk fluctuates during the day n early lactation it is 18 100 ml It can be incre mg daily [327] Liver a ly consumed in relatively small quantities they do not contribute much riboflavine to the diet A variety of yeast known as food yeast (*Torulopsis utilis*) contains as much as 9 mg of riboflavine per 100 grams [8] Meat and poultry muscle are fairly good sources but fish muscle is not Fresh raw peas and beans are fair sources White bread is a poor source In natural materials where there is little respiration riboflavine is found mainly in the free state, in tissues s enzyme (p 292) ing Since riboflavine is the ordinary processes of

cooking unless the medium is alkaline, although it is stated that the presence of cooking soda in a concentration of 0.12 per cent causes no destruction [127]. The solubility in water will result in some loss in the cooking water if this is not consumed. The loss of riboflavine from food cooked by boiling is from fifteen to twenty per cent [153]; slight losses also occur in pressure cooking [23]. With careful cooking up to practically one hundred per cent of the riboflavine present in the raw material may be retained. Losses from roasting vary from nil to twenty six per cent [24, 126, 153]. The losses are greater if the food is cooked while exposed to light, e.g. up to forty eight per cent in the case of eggs, milk and chops [117]. In the cafeteria cooking of food the loss of riboflavine is greater, from twenty two to forty five per cent [155].

Considerable quantities of riboflavine in milk may be destroyed if milk bottles are allowed to stand for any length of time exposed to the sun or bright daylight [309]. There is practically no loss of riboflavine in the pasteurization of milk [282]. In the curing of meat the losses of riboflavine are very small, e.g. about eight per cent. The loss in frying is about the same as in roasting (none to twenty three per cent) [24, 126]. Losses by drying or dehydration vary from nil up to fifteen per cent [156, 281]. Quick freezing has very little destructive effect on the riboflavine in foodstuffs [25, 157]. Meat kept in cold storage for fifteen days loses fifteen per cent of its riboflavine [24]. Canning and smoking have little effect on the riboflavine of foods, although in canning up to thirty per cent or more may be lost in the liquid used to can the food [26, 157, 281]. The average retention in the canning of vegetables, including the riboflavine in the water, is ninety per cent. Appreciable amounts of riboflavine are retained in the preservation of food by brining and salting [280]. Dehydration of vegetables results in a loss of twenty to forty per cent by the time the food reaches the table. Radio frequency or high frequency heating, which is used in the food industry particularly in America, results in little loss of riboflavine [116]. Not all the riboflavine in foods is available. Everson [120] has shown that there is a significant difference in the availability of the riboflavine found in various food sources. The riboflavine in live yeast is not available to any extent; the yeast must first be destroyed by boiling before the riboflavine in the cell can be utilized [154]. It appears that live yeast cells in their growth and reproduction enter into active competition in the gut for the riboflavine ingested in the food. If yeast is given as a dietary supplement it must be boiled first.

Riboflavine Content of Foodstuffs

Food	Description	Micrograms of Riboflavine per 100 grams (=3½ oz)
Cereals		
Barley	—	120 250
Buckwheat	—	80 160
Maize	Sweet, white or yellow	60 140
Oats	—	100 150
Rice	Unpolished	60 80
	Unmilled	120
	Milled and polished	47
Rye	—	140
Rye bread	—	40
Wheat	Whole grain	160 250
	Manitoba wheat	170
	Germ	480 1,500 (Av 800)
	Bran	500 600
	Whole grain bread	180
	White bread (70%)	70 60
	White bread (73%)	50

Food	Description	Micrograms of Riboflavine per 100 grams = 3½ ozs
Cereals—continued		
Wheat—*continued*	White flour (73%)	85
	White flour (82½%)	100
	National flour (85%)	140 200 (Av 150)
	National bread (85%)	100–200 120 130
	Flour (U S A enriched)	260–330
Spaghetti	—	80
Proprietary Cereal Foods [294]		
All Bran	Kellogg's	360–480
Bran Flakes	Post's (with added vitamins)	210 290
Cerevim	Lederle Labs (with added vitamins)	3,300
Corn Flakes	Kellogg's (with added vitamins)	80
	Post's (with added vitamins)	100
Force	—	160
Grape Nuts	With added vitamins	170–200
Quick Quaker Oats	—	140 180
Cream of Rice	—	80
Puffed Rice	With added vitamins	620
	Quaker Oats Co	60
Rice Krispies	Kellogg's (with added vitamins)	70
Shredded Wheat	—	140–190
Soya Wheat	—	270
Fruits		
Apple	—	10 50
Apricot	Fresh	40–75
	Dried	100
	Tinned	24
Avocado	—	90–150
Banana	—	50–75
Blackberry	—	60
Blackcurrant	—	140
Cherry	—	40
Date	—	45
Fig	Fresh	5
	Dried	45
Grape	Fresh	15–40
	Dried	80
Grape fruit	—	20–40
	Tinned	19
Guava	—	10 90
Melon	Cantaloupe	40–75
Orange	—	30 90
	Tinned	22
Peach	Yellow	60
	Tinned	2[illegible]
	Dried	200
Pear	—	[illegible]0 75
	Tinned	[illegible]0
Pepper green	—	50
Pineapple	—	5 55
Plum	—	30–45
Prune	Dried	160
Pomegranate	Juice	100
Pumpkin	—	45
Raisin	—	80
Raspberry	—	70
Strawberry	—	70
Squash	—	50
Tangerine	—	20–30
Water melon	—	15 60
Nuts		
Almond	—	300–670
Brazil	—	70
Cashew	—	190

Food	Description	Micrograms of Riboflavine per 100 grams = 3½ ozs
Nuts—continued		
Coconut	—	100 110
Pecan	—	106–300
Peanut	Raw	500 170 750
	Roasted	160–500
	Butter	160–320
Walnut	—	130
Vegetables		
Alfalfa	Dried	1 500
Arrowroot	—	60
Artichoke	—	30
Asparagus	—	120
Bean	Fresh	110 175
	Dried	Up to 750
	Baked	50
Beet	Tops	170 300
	Root	50
Broccoli	Entire plant	250
	Flower	240
	Leaves	450
Cabbage	—	50 57
	Dehydrated	380–470
Carrot	—	50 60
	Dehydrated	280
Cauliflower	—	105 130
Celery	—	40 100
Chard	—	130
Cucumber	—	25 90
Date	—	45 100
Eggplant	—	30–60
Endive	—	120–200
Grass	—	190
Kale	—	350–500
Lentil	Dried	315–400
Lettuce	—	45 150
Mango	—	50 100
Mushrooms	—	5 500
Onion	—	24–50
Papaya	—	150 180
Parsley	—	300
Parsnip	—	90
Pea	Fresh	150 200
	Dried	150–300
	Tinned	20 50
Peppers	Green	40 100
Potato	—	29–40
	Peeled and raw	50
	Chips	210
	Dehydrated	100
Pumpkin	—	80
Radish	—	30
Rhubarb	—	30
Spinach	—	230–400
Sprouts	—	60–75
Sweet potato	—	60 75
Soya bean	—	280 750
Tomato	—	40 60
Turnip	Root	30 65
	Greens	350–560
Water cress	—	250
Dairy Products		
Butter	—	10 37
Cheese	Camembert	830
	Cheddar	500 550
	Cottage	290
	Cream	140

Food	Description	Micrograms of Riboflavine per 100 gram = 3½ ozs
Dairy Products—continued		
Cheese—*continued*	Processed	430 570
	Roquefort	450
	Swiss	370
	Velveeta	550
Eggs	Whole	250–440
	White	250
	Yolk	520
	Dried	1,230
Margarine	—	0 6
Milk	Cow s average (new)	95 , 150
	,, range (new)	135–210
	,, pasteurized	119 206
	cream	140
	dried	1 500
	,, ,, (skimmed)	160–200
	,, evaporated	360–390
	Buttermilk	180
	Human	16–6"
	Goat	40–80
Meat Products		
Calf	Muscle	140 220
	Kidney	2 100
	Liver	3 300
Chicken	Muscle	70–260
Duck	—	230
Lamb	Heart	270–350
	Kidney	2 000
	Liver	3 300
	Muscle	250
Luncheon meat	Canned	210
Ox	Heart	900
	Kidney	2 000
	Corned beef	100
	Liver	3 000
	Muscle	180 350
	Brain	140
	Tongue	270
Pig	Bacon	130–300
	Muscle	180 240
	Ham	190
	Heart	900
	Kidney	2 100
	Liver	2 700
Rabbit	Liver	2 700–3 500
	Heart	550 1 330
	Kidney	1 310
	Muscle	60
Meat extracts	—	1,540–2,580 , 3 000–3 500
Sausage	Liver	1,120
Tripe	—	120
Turkey	—	190 240
Veal	—	260–280
Fish		
Carp	Muscle	40
Clam	—	120
Cod	Muscle	50
	Roe	900 1,130
Conger eel	Muscle	55
Crab	Flesh	350
Dogfish	Liver	370–540
	Roe	880
Haddock	Muscle .	165
	Roe .	1,420
Halibut	Muscle	185

Food	Description	Micrograms of Riboflavine per 100 grams = 3½ ozs
Fish—continued		
Herring	Muscle	104
	Roe	385
Kippers	—	300
Lobster	Muscle	130
Mackerel	Muscle	200–330
	Roe	1 140
Oyster	—	130–460
Prawn	—	110
Salmon	Whole	160–220
	Tinned	160 180
Sardines	Whole	190–330
	Tinned	110 180
Shrimp	—	160
Trout	Roe	680
Tuna	—	130
Turbot	Muscle	137
Miscellaneous		
Ale	—	390
Beer	—	50–170
Bemax	—	1 030
Chocolate	—	290
Cocoa	—	390
Coffee	—	70
Honey	—	0–40
Ice cream	—	150–190
Jam	—	20
Macaroni	—	80
Malt	—	560
Marmite	—	5 300–6 500 (Av 6 000)
Molasses	—	0 160
Royal jelly	—	820
Syrup	—	10
Tea	—	250
Yeast	Baker s	2 500–4 000
		3 500–8 000
		7 400
	Brewer s	1 800–3 000
		4 200
	dried	5 450
	D C L	5 000
	Torulopsis utilis (food yeast)	5 000 9 000
	Aluzyme	4 500

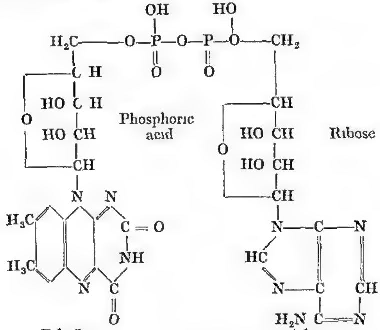
PHYSIOLOGY OF RIBOFLAVINE

Riboflavine and Flavoprotein Enzyme Systems *In 1932* Warburg and Christ [illegible] which was subsequent [illegible] [39] to consist of an en [illegible]

[illegible]

which was formerly the [illegible] part in tissue respiration Although there may be some doubt about the function of this enzyme in living tissues there is no question about the importance of other flavoprotein enzymes *containing* riboflavine such as D amino acid oxidase [35] xanthine oxidase succinic acid dehydrogenase [160 161] and diaphorase [16 [illegible] systems [illegible] linked to

an apoenzyme, which is a specific protein There are two different types of riboflavine co enzymes, namely, mononucleotides and dinucleotides The mononucleotide is a riboflavine phosphate and the dinucleotide a riboflavine adenine dinucleotide Many riboflavine co enzymes have been discovered, the properties of which are dependent on the protein apoenzyme with which the riboflavine containing prosthetic group is conjugated Riboflavine adenine dinucleotide is



Riboflavine Adenine

enzymes form part of a cycle for the trans ious reactions catalysed by these enzymes are

Enzyme	Hydrogen Donor	Hydrogen Acceptor
Warburg s yellow enzyme [31-33]	Reduced codehydro genases I and II	Oxygen Cytochrome *c*
Cytochrome *c* reductase	Reduced codehydro genase II	Cytochrome *c* Oxygen
Diaphorase I	Reduced codehydro genase I	Cytochrome *a* and *b*
Diaphorase II	Reduced codehydro genase II	?
Diaphorase [162]	Reduced codehydro genases I and II	?
D Amino acid oxidase [35]	D Amino acids	Oxygen
Aldehyde oxidase [70]	Aldehydes	Oxygen methylene blue
Xanthine oxidase [163]	Xanthine	Oxygen
Glucose oxidase	Glucose	?
Succinic dehydrogenase [160 161]	Succinic acid	?
Fumaric acid oxidase [165]	Reduced dyes	Fumaric acid

Warburg's yellow enzyme probably plays little part in cellular respiration because of its turnover number This is the number of times per minute that the enzyme can accept hydrogen from the substrate and transport it to the next acceptor in the series In one cycle the turnover number of Warburg s yellow enzyme is 50 compared with the figure of 8 000 for some other riboflavine enzymes

Riboflavine enzymes or flavoproteins form part of the system for the metabolism of carbohydrate This also involves codehydrogenases I and II,

also known as co-enzyme [illegible] I II which are complexes of nicotinic acid amide, adenine, ribose a[illegible] 220). The carbohydrate substrate, e g lactic acid, [illegible] [illegible]otinic acid enzymes, codehydrogenases I and II, [illegible] ro[illegible] [illegible]re compound. These in turn are oxidized by the riboflavine [illegible] The reduced riboflavine at the same time reduced to the dihydro compound The reduced riboflavine enzymes are then re-oxidized by specific reactions involving loss of hydrogen Thus the hydrogen may react directly with oxygen or it may react indirectly through the cytochromes *a*, *b* or *c* The following scheme has been suggested for the oxidation of lactic acid to pyruvic acid, before the oxidation of the latter to carbon dioxide and water [164]

I Lactic acid + lactic dehydrogenase + codehydrogenase I
  → pyruvic acid + reduced codehydrogenase I.

II Reduced codehydrogenase I + flavoprotein
  → codehydrogenase I + reduced flavoprotein

III Reduced flavoprotein + cytochrome *b*
  → flavoprotein + reduced cytochrome *b*.

In the chain of the oxidative change of lactic acid *via* pyruvic acid to carbon dioxide and water it is probable that all the three vitamins, riboflavine, nicotinic acid and aneurine are essential, and that the absence of any one of them may interfere with the process

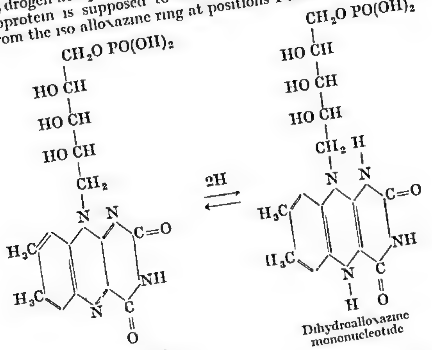
The general scheme for the oxidation of a metabolite through the agency of one of the nicotinic acid co enzymes and flavoprotein is as follows .

1 Substrate + enzyme + co enzyme → Oxidized substrate + enzyme + reduced co enzyme

2 Reduced co-enzyme + flavoprotein → co enzyme + reduced flavoprotein

3 Reduced flavoprotein + X → flavoprotein + reduced X

X is a hydrogen acceptor, e g cytochrome The reduction and oxidation of the flavoprotein is supposed to occur by the addition and removal of hydrogen from the iso alloxazine ring at positions 1 and 10



Alloxazine mononucleotide (Riboflavin phosphate)

Dihydroalloxazine mononucleotide

The fact that metabolically active tissues such as liver, kidney and heart muscle are rich in bound riboflavine, i e flavoprotein, suggests that riboflavine

plays the role of a respiratory catalyst [3] It probably plays a part in the metabolism of muscle as even minute quantities (e g 10^{-5} mM per litre) can increase the work done by a contracting isolated muscle [285] According to Leeman and Pichler [170] the riboflavine content of various parts of the brain is directly proportional to the rate of respiration A fall in the alloxazine adenine dinucleotide content of the muscle liver and brain occurs when an animal is subjected to hæmorrhagic shock [305] The brain is the first tissue to suffer A fall in the cocarboxylase content also occurs (p 258)

Riboflavine may be involved in the formation of glucose and glycogen in the body If rats deficient in riboflavine are exposed to a low oxygen tension they do not increase their liver glycogen to the same extent as normal animals a normal response occurs if they are injected with riboflavine before the test The blood glucose is also lower than normal in anoxic rats [244] According to Drilhon [249] a rise of blood sugar occurs in riboflavine deficient rats a fall is said to occur when riboflavine is injected into a normal rat [254]

Phosphorylation Riboflavine must be phosphorylated before it can possess vitamin activity This phosphorylation may occur as soon as it is absorbed from the intestinal wall since preparations of intestinal mucosa can bring about the phosphorylation of riboflavine in presence of phosphates [36] The phosphorylation of riboflavine probably occurs in the liver as well [3 98] and human blood cells can synthesize both the phosphate and dinucleotide from riboflavine *in vitro* and *in vivo* [178]

According to De Preux [3] the liver not only stores riboflavine but also

micro organisms including those in the rumen of herbivorous animals can synthesize riboflavine [106 172] Synthesis also occurs in the cæcum of the rat but only under certain dietary conditions Although riboflavine deficiency does undoubtedly occur in man it has not always been reported in subjects

It has for example been reported in subjects

f riboflavine daily while others have failed

less than 1 mg daily (pp 302 303) The

onditions intestinal micro organisms can

synthesize riboflavine in man has been considered to account for this discrepancy Najjar Holt and their colleagues [297] have recently shown that in man the urinary and fæcal excretion of riboflavine may exceed the intake The fæcal excretion in twelve subjects kept under observation for twelve weeks varied from 200 to 600 micrograms daily although the intake was only 60 to 90 micrograms a day The most likely explanation of this is that biosynthesis of riboflavine by the intestinal flora occurs

after treatment with riboflavine It is possible that streptomycin is excreted into the gut and destroys the organisms that synthesize riboflavine If the intake of the latter is low symptoms of deficiency might then occur

Riboflavine Deficiency in Animals In the dog riboflavine deficiency causes bradycardia cardiac arrhythmia yellow mottling of the liver degenerative changes in the central nervous system collapse and finally coma [49 50] These symptoms which occur very rapidly can be prevented by the administration of riboflavine Pathologically the most striking lesions occur in the liver and central nervous system both of which are profoundly affected by changes in carbohydrate metabolism Blood glucose and chloride fall and there is an eosinopenia [328] The condition resembles that following adrenalectomy

In the rat deprived of riboflavine the weight remains stationary, the animal develops alopecia and an eczematous condition of the skin affecting specially the nostrils and the eyes, the rims of which become denuded of hair [123], and there is dullness of the cornea, blepharitis and conjunctivitis, the eyelids being stuck together with a serous exudate [51]. Water retention also occurs in the riboflavine deficient rat [46]. There is some evidence that the resistance of the rat to infection (e.g. typhus and leprosy) is lowered [52, 135] and that fertility is seriously impaired [136]. Increasing the fat level in a ration low in riboflavine has a deleterious effect on the growth of young rats; the administration of adequate amounts of riboflavine completely corrects this deficiency [113]. These and other observations suggest some relationship between riboflavine and fat metabolism [124]. Rancid fat also accentuates the signs of riboflavine deficiency [333]. It has been shown by Warkany [61] that riboflavine deficiency in the rat may result in gross malformation of the offspring, e.g. syndactyly, microphthalmos, brachygnathia and cleft palate. Riboflavine is also essential for the nutrition of the pig, calf and monkey. Acute deficiency in the latter produces anaemia and a freckled dermatitis on the face, extremities and groin [238].

Riboflavine and the Eye. Conjunctivitis and keratitis occur in animals on riboflavine free diets within seven to eight weeks, followed by a dullness of the eyeball and finally, according to some observers, opacity of the lens [38]. These eye lesions are no doubt due to defective metabolism in the lens and cornea following lack of the respiratory enzyme flavoprotein. The normal epithelium of the cornea of the rat has a high oxygen uptake, which falls if the animal is put on a diet deficient in riboflavine [296]. This fall in the oxygen consumption is probably due to necrosis of the epithelial cells of the cornea. Evidence for the role of riboflavine deficiency in the production of cataract is very conflicting. Day and other observers [38, 39] have described its occurrence in several species deprived of riboflavine, and its arrest in eighty nine per cent. of the animals treated by the administration of riboflavine in doses of 120 micrograms twice weekly. Bourne and Pyke [65] however, could only induce cataract in twenty to thirty per cent. of their animals by depriving them of riboflavine, and Gyorgy [66] observed no cataract in five hundred rats treated in this way. The problem was reinvestigated by Eckhardt and Johnson [67], who produced cataract in only two out of twenty three rats kept on a diet poor in riboflavine and rich in galactose. The subsequent administration of riboflavine did not prevent the cataract from forming in the second eye. Baum and his co-workers [122] state that rats on diets completely devoid of riboflavine do not suffer from cataract; they only do so if minute amounts of riboflavine are present, although a normal intake is non-cataractogenic. Riboflavine does not arrest the progress of lens opacities in the human eye [68].

The existence of a flavoprotein and a cytochrome-cytochrome oxidase enzyme system in the lens has been demonstrated [332]. These respiratory enzymes are more concentrated in the epithelium than the cortex or nucleus. The ratio of lactate and pyruvate concentrations in the various parts of the lens suggests that the metabolism of the epithelium is strictly aerobic, while that of the cortex and nucleus is anaerobic.

Wolbach and Bessey [40, 41] state that corneal vascularization is an early, specific and most reliable criterion of ariboflavinosis, or riboflavine deficiency, in rats. They considered that this vascularization is a response to the respiratory needs of the corneal epithelium, in which oxidation occurs through the mediation of flavoprotein.

Riboflavine has been found in the retina of many species. Adler and Euler [181] consider that it plays some part in a light sensitive reaction because of the fluorescence of free riboflavine. They state that free riboflavine occurs in the fish retina and that it is therefore possible that in fish riboflavine acts as a photosensitizer by absorbing short wave light and transmitting it

as light of longer wave length. In the mammalian retina, however, Pirie [180] was unable to demonstrate much free riboflavine; it is nearly all bound as riboflavine adenine dinucleotide, which is light stable. There is therefore no evidence that riboflavine acts as a photosensitizer in the mammalian retina. Heiman [176] believes that riboflavine is essential for the visual act and may be a factor in cone vision by functioning in the flavoprotein oxidation-reduction system and by its power to intensify weak light. He accepts the unproven assumption that riboflavine acts as a photosensitizer in the retina. This, incidentally, is accepted by many writers as an established fact, although the only evidence for this is the work of Adler and Euler [181] on the frozen fish retina. According to Philpot and Pirie [250], practically all of the riboflavine in ocular tissues is present as the adenine dinucleotide, and is therefore not affected by light. They find that there is very little riboflavine in the cornea (0.2 microgram per gram), but much more in the lacrymal gland (6.5 microgram per gram). They therefore suggest that the riboflavine of the cornea is derived from the lacrymal secretions by diffusion.

Dimness of vision, impairment of visual acuity, photophobia, lacrimation, inability to see in a dim light, visual fatigue and corneal vascularization have been described as clinical manifestations of riboflavine deficiency (p. 313). Kimble and Gordon [44] have observed that individuals showing poor dark adaptation and a low vitamin A blood level did not improve with vitamin A alone, but responded to the administration of both vitamin A and riboflavine. This observation may mean that riboflavine plays some part in the visual act; on the other hand it may play a part in the absorption and utilization of vitamin A. Pock-Steen [45, 78] observed that in 109 patients with incipient sprue and frank sprue many suffered from eye symptoms, the principal one being reduced visual acuity in dim light. This "twilight blindness" or "aknephascopia" was considerably relieved by riboflavine but not by vitamin A. The same observer also states that patients suffering from riboflavine deficiency show poor dark adaptation [158].

Riboflavine and Hæmatopoiesis. Miller and Rhoads [43] were able to produce in dogs a syndrome similar to sprue, a disease associated with a macrocytic anæmia, by feeding diets deficient in riboflavine. György and his co-workers [48] also noted that riboflavine causes a definite increase in hæmoglobin production above the basal level when fed to dogs in which anæmia was produced by a deficient diet. Rats kept on a riboflavine-deficient

which appears to be sufficient for adequate hæmoglobin production. The anæmia produced by a deficiency of riboflavine was of the microcytic hypochromic type; when small amounts of riboflavine were given to the dogs after slight bleeding the anæmia was normocytic and hypochromic.

ing the size of new blood

deaminated by an enzyme

it is possible that ribo

and therefore possibly for

the formation of blood proteins. Although Waisman [238] has proved that anæmia occurs in monkeys fed on diets deficient in riboflavine, in man there is no substantial evidence that riboflavine is essential for hæmatopoiesis; the anæmia in monkeys is not completely corrected by riboflavine and only responds completely when pteroylglutamic acid is given [334]. Keys [28]

Riboflavine and Tumour Formation Rats fed butter yellow (*p* dimethyl amino azobenzene) or treated with it externally develop cancer of the liver This is prevented by giving the rats riboflavine which appears in such cases to have an anti carcinogenetic effect [183] This is of some interest as the susceptibility of natives in the Far East and South Africa to primary carcinoma of the liver may be due to a dietary deficiency The native diet in areas where these primary liver carcinomata are found is deficient in the vitamin B complex and riboflavine The concentration of riboflavine in tumour tissue is low *in comparison with that of normal tissues* [184] This is in keeping with the view that cancerous tissues have a deficient aerobic oxidation system A diet rich in riboflavine increases the resistance to tumour formation in rats [258]

Riboflavine and Nitrogen Metabolism In animals and in man there is a close correlation between nitrogen and riboflavine metabolism An increase in the amount of protein ingested increases the riboflavine content in the liver of the rat [221] In man the excretion varies inversely as the protein intake [234] It would appear that riboflavine is released and excreted when reserve protein is depleted and stored when it is replenished Thus normal persons will retain more than fifty per cent of ingested riboflavine when in nitrogen equilibrium and less than fifty per cent when in negative nitrogen balance [330] If the latter is excessive as after trauma or surgical procedures and in untreated diabetes more riboflavine is excreted than is ingested but post operatively when the balance is positive riboflavine is retained [144 330]

Riboflavine Metabolism and Other Vitamins Other vitamins appear to play some part in riboflavine metabolism Thus the storage of riboflavine in the liver which occurs in a normal animal after injecting the vitamin is considerably influenced by the tissue stores of aneurine [173] The riboflavine content of the tissues also falls considerably mainly because of rapid excretion and poor absorption in animals suffering from aneurine deficiency [174] It has been argued that these changes in riboflavine metabolism are observed only in the terminal stages of aneurine deficiency and appear to be unspecific [175] According to Delachaux [30] the metabolism of riboflavine is linked with that of aneurine since administration of large quantities of the latter results in an increased excretion of riboflavine and *vice versa*

Pantothenic acid appears to have a direct and specific function as part of the *mechanism* whereby *riboflavine* is stored in the liver after the ingestion of food [173]

Chemically Induced Riboflavine Deficiency Vitamin deficiency can be induced in animals experimentally by feeding compounds structurally related *to the vitamin The symptoms of riboflavine deficiency* in animals have been reported following the administration of 2 4 dinitro 7 8 dimethyl 10 ribityl 5 10 dihydrophenazine [284] and galactoflavine in which the ribityl side chain of riboflavine is substituted by a dulcityl [53] Inversion of the hydroxyl *groups in the side chain forms d araboflavine* which is also a ribo flavine antagonist in rats [58] It is supposed that there is an active compe

ition bet essential enzyme reaction

nd that 2 Dichloro 4 5 diamino

enzene can [illegible] by growing cultures of

B megatherium [326] It is not an analogue but 1 2 dimethyl 4 5 diamino

benzene may be a precursor of riboflavine and the dichloro analogue may exert its selective action by inhibiting the biosynthetic process in which *riboflavine is formed*

Pharmacology of Riboflavine The pharmacology of riboflavine has been studied by Unna and Greslin [185] who state that in rats 10 grams per kilo and in dogs 2 grams per kilo orally fail to produce any toxic effects The low *solubility* of the vitamin prevents its absorption from the gastro intestinal tract in amounts sufficient to produce toxic effects Likewise the subcutaneous injection of doses of 5 grams per kilo produces no toxic

effects [81] The sodium salt is more soluble and 300 mg of this intraperitoneally is lethal in the rat Death occurs within two to five days with signs of anuria due to renal blockage with crystalline concretions The daily administration of 10 mg of riboflavine to rats and 25 mg per kilo to dogs for a period of four months produces no sign of toxic manifestations The metabolism circulatory and respiratory systems and isolated smooth muscle organs of the animals are unaffected

Absorption Storage and Excretion of Riboflavine Riboflavine is absorbed from the intestine and gastric hydrochloric acid is probably needed for its absorption Phosphorylation is thought to occur in the intestine although it can take place in the liver blood and tissues Riboflavine is not readily phosphorylated or absorbed in patients with gastro intestinal disease it is only utilized in such patients if it is injected [134] The bulk of the riboflavine in the body is stored in the liver heart and kidneys Rats maintained on a low protein diet are unable to store the normal amount of riboflavine in the liver [287] On the other hand deficiency of aneurine causes increased storage of riboflavine in the liver [304] If there is a considerable increase in intake there is only a slight increase in the amount stored in the liver and even if the animal dies for want of riboflavine the quantity present in the liver kidney and heart is only about a third of normal [54] The body therefore appears to cling tenaciously to its stores of riboflavine Human blood contains about 0 5 microgram per gram [111] Much of it is in combination with the euglobulin of the plasma [300] The free riboflavine in plasma is 0 84 ± 0 71 micrograms per 100 ml [335] Destruction of riboflavine in the body occurs but to what extent is unknown If riboflavine is injected there is an immediate concentration in the liver [57 173] The riboflavine content of the latter also increases during digestion and assimilation being mobilized from other tissues [173]

When given intravenously the bulk is excreted into the small intestine particularly the duodenum from which it is reabsorbed [186] It is largely destroyed in the large intestine and some destruction also occurs in its passage through the kidney

Certain factors influence the excretion of riboflavine Thus in man the administration of large doses of aneurine e g 1 to 10 mg over a period increases the excretion of riboflavine in the urine although such dosage does not produce clinical riboflavine deficiency [187] Chronic aneurine deficiency increases urinary excretion considerably in rats [288] The protein intake also influences riboflavine excretion [188 316] An increased protein intake produces a diminished excretion and increased retention of riboflavine If the carbohydrate/fat ratio of the diet is low less riboflavine is excreted [316] The excretion of riboflavine in rats is increased in experimental hyperthyroidism [189] and in man the excretion falls after exercise [286] Caver and Cody [110] found no difference in the riboflavine excretion of patients suffering from acute infections and various chronic diseases except in patients with peptic ulcer who consumed large quantities of milk Increased retention and decreased excretion occur as a result of haemorrhage infection and trauma (fractures burns) [144 171 294] and in pregnancy [172] The diminished excretion after severe burns parallels the severity of the injury and the upset of nitrogen metabolism [171]

Riboflavine is also excreted in the sweat The estimates of the amount lost in this way are variously given as from 5 to 180 litre and 10 micrograms per hour [193 195] figure of 120 micrograms per litre this only of a good intake and is therefore a negligible loss According to Sargent Robinson and Johnson [278] there is no free riboflavine in sweat

Riboflavine is excreted into the milk on a daily intake of 3 mg about 0 3 mg or ten per cent is excreted in the milk [112] After delivery it is low (65 micrograms in twenty four hours) but increases later [172]

If the diet is adequate riboflavine is excreted in the urine in the form of uroflavin a pigment almost identical with it in composition properties and vitamin activity There is a fairly general dependence of uroflavin excretion upon riboflavine intake [109]

Emmerie [55] who has studied the riboflavine excretion in man estimates that the urinary output of flavin is from 819 to 1 250 micrograms daily Strong [111] puts it at from 500 to 800 micrograms a day on a normal diet and 50 to 150 micrograms on an intake of 1 to 2 mg a day This agrees fairly closely with the figures of Feder Lewis and Alden [302] who found that the daily excretion was 500 to 1 000 micrograms with an average of 800 micro grams on a daily intake of 2 to 3 mg According to Conners Eckhardt and Johnson [192] the average riboflavine excretion of a group of normal subjects was 1 032 micrograms Keys and co workers [273] state that on an intake of 0 31 mg per 1 000 calories daily, the average daily excretion is twelve per cent of the daily intake On an intake of 1 6 mg daily the excretion is twenty five per cent of this it drops to ten per cent on a daily intake of 1 1 mg [331] An increased intake is reflected in an increased excretion when the intake is considerably decreased there is an increased retention and decreased excretion In pregnancy on a low intake e g 1 75 mg daily the urinary excretion is about seventeen per cent of the intake this rises with an increased intake and reaches seventy to eighty per cent on an intake as high as 7 mg daily [167] For the first four days after birth excretion exceeds intake [312]

Riboflavine deficiency cannot be detected by a single determination of a twenty four hour specimen of urine It fluctuates with the food intake and

workers

hundred

Actually

it is possible by placing human subjects on a diet low in riboflavine to depress the excretion to zero without evidence of clinical riboflavine deficiency [126] Coryell and her co workers [325] state that the fasting hour excretion in boys is 45 micrograms and 38 micrograms in girls

Axelrod Spies and Elvehjem [109] have carried out saturation tests on human beings with riboflavine employing intravenous injections of 200 to 400 micrograms of riboflavine per kilo of body weight but they were unable to detect any correlation between the amount of the test dose of riboflavine retained and the daily urinary riboflavine excretion Sebrell and co workers [119] state that in a condition of riboflavine depletion there is a close relationship between riboflavine intake and excretion Axelrod Spies and Elvehjem [109] observed that injected riboflavine was rapidly excreted in the urine from thirty to forty per cent being excreted after a test dose of 200 micrograms per kilo of body weight They were also able to produce an uncomplicated riboflavine deficiency in the dog in which the degree of retention of a test dose of riboflavine was found to be a measure of the riboflavine deficiency [125]

Another saturation test to detect human riboflavine deficiency has been devised by Najjar and Holt [126] They inject 1 mg of riboflavine intra enously after an overnight fast and follow the urinary excretion for half ourly and hourly periods over four hours following the injection Normally marked excretion occurs in the first half hour and during the second and ıbsequent hours the excretion falls off rapidly approaching that of the ııtial control period From thirty two to seventy two per cent of the dose retained In riboflavine deficiency the excretion is much less from ıghty one to ninety three per cent being retained in the four hour test s the weight of the individual also influences the excretion Najjar and Holt ıggest a dose of 0 016 per kilo rather than a flat dose of 1 mg for all subjects oryell and her co workers [325] found the mean four hour urinary excretion fter a test dose of 1 mg to be 351 micrograms for boys and 344 micrograms

Urinary Excretion of Riboflavine before and after Test Doses

Author	Subjects	Daily Dietary Intake of Riboflavine mg	24 Hours Urinary Excretion mg	Test Dose	Excretion After Test Dose
Emmerie [55 56]	Normal	?	0 952 0 885	5 71 mg 4 24 mg	3 28 mg 1 20 mg
Sebrell *et al* [119]	Normal	2 54–3 68	0 793 1 265	5 mg	50 80%
	Ariboflavinosis	0 5	0 024–0 119	2 mg	Excretion rose from 34 micrograms to 1 48 mg on 20th day
Axelrod *et al* [109]	Ariboflavinosis	0 3 per 2 000 cals	0 058–0 091	0 2 0 4 mg per kg i v	30–40% excreted after 1 hour
	Normal	?	0 236–0 270		?
Najjar and Holt [126]	Normal	?	0 3–0 68 in 4 hours	1 mg i v	32 72% retained
	Ariboflavinosis	Very low	0 074–0 194 in 4 hours	1 mg i v	81 93% retained
Swaminathan [190]	Normal	1 2 1 5	0 32 0 36	1 10 mg orally	80–85% excreted
Swaminathan and Verma [191]	Ariboflavinosis	0 4–0 5	0 05 0 2	5 mg	10 20% excreted
Conners *et al* [192]	Normal	?	1 03	1 mg i v 5 mg i m	38 6% retained 21 5% retained
	Patients with rosacea keratitis	?	0 62	1 mg i v 5 mg i m	21 3% retained 47 5% retained
Williams *et al* [200]	Ariboflavinosis	0 76	0 06–0 15	2 mg s c	0 103–0 348 mg after 4 hours
	Normal	1 3	0 46–1 8		0 311 1 155 mg
Harris and Scoular [159]	Normal	1 25 2 5	36 51% of intake	5 mg	24–44% excreted in urine
Goth [243]	Normal	?	—	3 5 mg	30–50% of test dose in 2 hours
	Deficient	?	—	3 5 mg	Less than 30% of test dose in 2 hours
Sastri *et al* [316]	Normal	0 76–1 91	0 4 1 977	1 mg	4 4–85 3% excreted
Ruffin *et al* [62]	Normal	?	0 8	5 mg	3 3 mg in 24 hours
	Deficient	?	0 4	5 mg	Less than 2 2 mg in 24 hours

for girls The excretion of riboflavine in a group of institution children in America was 0 032 to 0 055 mg hourly after a test dose of 1 mg it rose to 0 273 to 0 387 mg hourly [166]

Feder Lewis and Alden [302] state that saturation tests give no more information on the level of riboflavine nutrition than do single riboflavine estimations on a fasting morning specimen of urine

The results of test doses in the urinary excretion of riboflavine are given above

The amount of riboflavine excreted in the fæces is determined largely by the degree of intestinal synthesis which is affected by the nature of the diet but is largely independent of its riboflavine content In man the fecal excretion is greater than the urinary, and on a low intake (e g 0 36 mg a day) three times the intake [63] The nature of the diet is important When human volunteers were kept on a diet containing 1 33 mg of riboflavine a day, the riboflavine being alternatively provided by a natural diet and a

synthetic one the fæcal excretion on the former was eighty four per cent of the intake while on the latter only twenty eight per cent [64] Carbo hydrates such as dextrin corn starch and lactose favour the development of the intestinal bacteria that synthesize riboflavine [69] The work of Czaczkes and Guggenheim [71] suggests that on a low protein diet the ability to store riboflavine in the tissues is reduced and urinary excretion is increased The fæcal excretion of riboflavine appears to be proportional to the number of viable bacteria in the fæces and presumably to the amount of riboflavine they synthesize According to Oldham and co workers [72] the urinary excretion of riboflavine in man varies inversely with the nitrogen balance When the balance is positive forty to sixty per cent is excreted when it is negative it is only seven per cent The fæcal excretion is unaffected

To what extent the riboflavine in the fæces is absorbed is unknown According to Najjar and Barrett [108] most of the riboflavine synthesized by intestinal bacteria is retained by the bacteria and excreted in the fæces the riboflavine is only liberated after the bodies of the bacteria disintegrate Fæcal excretion varies considerably from person to person although the amount excreted by any one individual is fairly constant despite dietary variations [283] According to Sastri and his co workers [316] considerable quantities of riboflavine may be derived from the fæces the urinary excretion in some cases being as much as 250 per cent of the intake in the food

HUMAN REQUIREMENTS OF RIBOFLAVINE

The requirements of riboflavine have been calculated from (*a*) an analysis of normal diets (*b*) from the intake of riboflavine by subjects suffering from ariboflavinosis (riboflavine deficiency) (*c*) from studies with experimental diets (*d*) from excretion studies

Dietary surveys have been made by a number of observers [59 104 197 275] According to these surveys the riboflavine intake of the average adult varies from 1 1 to 2 mg although a good many persons in low income groups probably receive no more than 0 7 to 0 8 mg A survey of the diets of English munition workers made in 1943 revealed that the average daily intake of riboflavine was 1 1 to 1 3 mg [207] If a diet deficient in riboflavine is consumed over a period symptoms of ariboflavinosis develop (p 305) Many observers have attempted to note the dietary intake of riboflavine that just produces such symptoms and they assume that a figure slightly above this represents the minimum riboflavine requirement [119 277 290] Some workers state that deficiency symptoms do not occur on intakes of 1 5 mg or more daily but appear on an intake of 0 3 to 0 5 mg [119 198] although this has been disputed [199]

Experimental diets have been administered to volunteers to see if symptoms of ariboflavinosis appear [60 119 199 200 227 273] Deficiency symptoms have been reported on diets containing from 0 5 to 0 55 mg daily [60 119 260 331] although some workers have not confirmed this [168] Williams and his colleagues [200] and Keys and his co workers [273] failed to observe deficiency symptoms on a daily intake of 0 31 to 0 35 mg of ribo flavine per 1 000 calories or 0 9 mg for the sedentary man In Keys study tests included work on a treadmill an anaerobic work test a glucose tolerance test tests of muscle power and psychomotor tests Blood studies and slit lamp examination of the eye showed no abnormality The average urinary excretion was twelve per cent of the intake Friedemann and his co workers [227] also kept volunteers on a daily intake of 0 95 mg riboflavine for five to seven months without observing any signs of deficiency All the subjects remained in good health According to Hagedorn [168] there is no correlation between the riboflavine intake and the appearance of lesions characteristic of ariboflavinosis In all these studies it is possible that the period of deprivation was too short Horwitt and co workers [260] produced the typical

facial and scrotal lesions of ariboflavinosis in subjects kept on a daily riboflavine intake of 0 5 mg for periods ranging from four to ten months It would appear that an intake of 0 5 mg of riboflavine daily is a critical level at which deficiency symptoms may occur after several months

Excretion studies have been employed to calculate the riboflavine requirements of man [55, 56 111, 119, 169, 200, 203, 260, 283, 291] The daily intake of riboflavine that causes neither a progressive decrease nor increase in excretion when graded amounts are administered to subjects on a basal diet is taken to be the normal requirement Some workers accept an arbitrary figure for the excretion of a test dose of riboflavine as evidence of a satisfactory intake Oldham and her co workers [291] accept an excretion of twenty per cent of a test dose in fours as adequate

Author	Daily Requirement Calculated from Excretion
Snyderman, *et al* [169]	
Oldham *et al* [291]	years)
Sebrell [119]	
Williams *et al* [200]	
Strong [111]	1 2 mg minimum (adults)
Brewer *et al* [203]	1 3 1 5 mg per 2 100–2 300 cals (adults)
Horwitt *et al* [260 331]	1 1–1 6 mg (adults)

These figures range from 1 to 3 mg for an adult, the most recent figures being between 1 and 1 5 mg This is approximately the range found by other methods

The following are the recommended daily allowances of riboflavine suggested by the Food and Nutrition Board of the National Research Council, U S A [1948]

	Daily Riboflavine Requirements in mg	Calories
Man (70 kg)		
Moderately active	1 8	3,000
Very active	1 8	4,500
Sedentary	1 8	2,400
Woman (50 kg)		
Moderately active	1 5	2,400
Very active	1 5	3,000
Sedentary	1 5	2,000
Pregnancy (latter half)	2 5	2,400
Lactation	3 0	3,000
Children up to 12 years		
Under 1 year	0 6	110/2 2 lb
1– 3 years	0 9	1,200
4– 6 ,,	1 2	1,600
7– 9 ,	1 5	2 000
10 12 ,	1 8	2 500
Children over 12 years		
Girls, 13–15 years	2 0	2,600
16–20 ,,	1 8	2,400
Boys 13–15 ,,	2 0	3 200
16 20 ,,	2 5	3,800

These figures for the riboflavine requirements of man may need revision in the light of work on the human biosynthesis of riboflavine (p 295) To what extent this normally occurs in man is not known nor is it known how much is absorbed

The Nutrition Committee of the British Medical Association (1950) has suggested the following daily allowances of riboflavine

	Daily Allowance of Riboflavine in mg
Children up to 1 year	0·6
„ 2– 6 years	0·9
„ 7–10 „	1·2
Adult male doing very light work (2,250 cals.)	1·4
„ „ „ light work (3,000 cals.)	1·8
„ „ „ medium work (3,500 cals.)	2·1
„ „ „ heavy work (4,250 cals.)	2·6
„ „ „ extra heavy work (5,000 cals.)	3·0
Female 11–14 years	1·6
„ 15–19 „	1·5
„ 20+ „	0·9
„ adult (2,000 cals.)	1·2
„ doing light work (2,500 cals.)	1·5
„ „ heavy work (3,000 cals.)	1·8
„ very heavy work (3,750 cals.)	2·2
Pregnancy, first half	1·5
„ second half	1·6
Lactation	2·1

It is assumed from animal studies that the daily requirement of riboflavine in man depends upon sex, age, degree of physical activity and calorie intake, and is increased in pregnancy, lactation and fever. Requirements are increased as a result of physical exercise [47] and a high fat diet [57]. Ingestion of large amounts of nicotinic acid by patients on a deficient diet is said to increase riboflavine requirement [115]. Mills [202] has shown that the requirements of riboflavine are independent of environmental temperature (cf. aneurine). In traumatic conditions (fractures) and after severe burns the riboflavine requirement may be increased as there is marked retention and diminished excretion of the vitamin [144, 171].

THE RIBOFLAVINE DEFICIENCY SYNDROME. ARIBOFLAVINOSIS DISEASES ASSOCIATED WITH RIBOFLAVINE DEFICIENCY

Historical. The essential features of riboflavine deficiency, or ariboflavinosis, were described as far back as 1911 by Stannus [84], who clearly described a group of symptoms including soreness of the tongue and lips, with a sodden excoriated condition at the angles of the mouth (which he termed angular stomatitis) and palpebral fissures, and a characteristic lesion at the free border of the prepuce, the vulva and anus, together with a dermatosis of the scrotum often spreading to the skin of the adjacent thighs. Stannus described the smooth tongue devoid of papillæ and denuded of epithelium—*the so called magenta tongue of ariboflavinosis*—some twenty five years before riboflavine was discovered. He knew that these lesions were the result of dietary deficiency but beyond that he could not go as the vitamins had not then been differentiated. Shortly afterwards, in 1918, Scott [139] described a condition among Jamaican coolies that showed some of the symptoms described by Stannus and in addition a central neuritis, photophobia, indistinctness of vision, ulceration and discharge of the eyelids and a burning sensation. A few years later Goldberger and Tanner [245] also gave an account of these symptoms, which they produced experimentally in American prisons. They noted that the condition was cured by autoclaved yeast. In 1930 Fitzgerald Moore [246] confirmed the observations of previous workers and added retrobulbar *neuritis as part of the syndrome* (p. 315),

which he stated was common in West Africa West Indies and Malay and was responsible for the blindness of thousands of natives The dietary origin of the syndrome was confirmed by curing it with marmite a source of the vitamin B complex Moore [247] later showed that neither vitamin A nor nicotinic acid deficiency played any role in the ætiology of the syndrome Landor and Pallister [248] completed the clinical picture by describing the

marmite or liver In 1938 Sebrell and Butler [82] published their observations on induced riboflavine deficiency in man

Incidence of Ariboflavinosis Judging from the literature riboflavine deficiency is relatively rare in the British Isles It has been described here by Duckworth [138] Deeny [204] and Scarborough [205] but the number of cases recorded is few In certain parts of America particularly the Southern States it is stated to be common Thus Goldsmith [206] states that forty to sixty seven per cent of hospital patients in Louisiana show some evidence of riboflavine and nicotinic acid deficiency A medical survey of Newfoundland in 1944 revealed that riboflavine deficiency was comparatively common in that country [34]

According to Farber and Miller [223] riboflavine and nicotinic acid deficiency is common in tuberculous patients Lesions stated to be characteristic of riboflavine deficiency were observed in twenty five per cent of 400 patients in an American sanatorium Riboflavine deficiency is very common among the Chinese according to Hou [105] and from the literature it would seem to be common in India [85 198 235 236] and among African natives [84 143 201] Braun Bromberg and Brzezinski [129] have reported the presence of riboflavine deficiency among pregnant women in Israel , the condition improved after delivery and after administration of riboflavine

Jones and his co workers [277] noted that in a camp of over 10,000 men of mixed races in North Africa some 1 746 or seventeen per cent showed

Clinical Manifestations attributed to Riboflavin Deficiency (Ariboflavinosis)

Lips	Tongue	Skin		Eyes	Neurological
		Face	Elsewhere		
Cheilosis Angular stomatitis Vertical fissuring Crusting of lips Stomatitis Burning of lips Redness and desquamation of lips	Glossitis Magenta tongue Flattened papillæ and epithelium Burning tongue Fissured tongue Dysphagia Patchy oval desquamation	Seborrhœic dermatitis on alæ nasi nasolabial folds eyelids and ears Shark skin eruption Comedones Capillary dilatation Flushing of face	V I		
				Corneal vascularization Retrobulbar neuritis Blepharospasm Opaque corneal infiltrates Nebulæ Corneal epithelial dystrophy Rosy eyes Rubeosis Mydriasis Iritis Pigmentation of the iris	Vertigo Tremor Clonic contractions Dysdiadokokinesis Dysmetria Mental apathy

signs of ariboflavinosis. The outstanding features were stomatitis and cheilosis, the lesions responded to riboflavine therapy in a selected group of cases

Lesions of Lips. In 1938 Sebrell and Butler [82] induced a deficiency syndrome in eighteen women, who were given a diet complete in all respects, except that it was deficient in the vitamin B complex. Daily supplements

FIG 85 Lips and Tongue in Ariboflavinosis The case shows considerable erosion at the corners of the mouth (angular stomatitis) The lips are denuded, red and cracked and covered with crusts of blood The tongue is fiery red and cracked

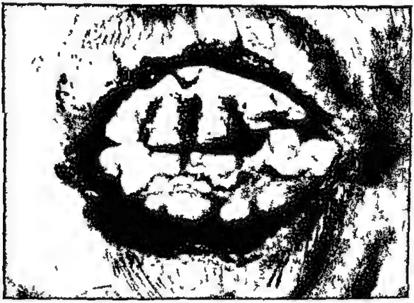


FIG 86 Ariboflavinosis, showing Cheilosis A case showing cheilosis with denuded lips and blood crusts There is also considerable hyperplasia of the gums suggesting scurvy

of aneurine were given to eliminate any deficiency symptoms of this vitamin Within ninety four to 130 days ten of the women developed cheilosis,* which began as a pallor of the mucosa of the lips in the angles of the mouth, and was followed by maceration and bilateral transverse fissures (Fig 85) The lesions, which remained moist and became covered with a honey-coloured crust

* There has been some confusion over the term cheilosis and angular stomatitis, which some writers use interchangeably Cheilosis is a lesion of the vermilion of the lip, angular stomatitis the lesion at the muco cutaneous junction at the corner of the mouth

(Fig 86) were identical with those described by Stannus [84] under the name angular stomatitis and by earlier workers The fissures extend 1 to 3 mm on to the mucous membrane of the mouth and up to 10 mm on the skin They are usually shallow but may be 0 5 mm deep The lips became abnormally

vertical fissuring

86) Sebrell and

a mildly erythe

matous base in the nasolabial folds on the alae nasae in the vestibule of the nose and on the ears This syndrome was termed ariboflavinosis by Sebrell and Butler and hypo riboflavinosis by Stannus [240] It is identical with the *pellagra sine pellagra* and the *formes* fr...

and others between 1911 and 1935

and Butler were described by Jolliffe

Sebrell and Butler treated four of the ten volunteers with 1 to 2 mg of synthetic riboflavine daily for three to ten days and then with doses corresponding to 0 025 mg per kilo of body weight This was later increased to 0 05 to 0 075 mg per kilo [87] All the lesions disappeared in from five to forty seven days but in controls treated with 100 mg of nicotinic acid daily the cheilosis was definitely worse It cleared up in the controls however when they r kilo of body weight One woman had t and cheilosis After thirty days treat igrous lesions healed but not the cheilosis which became worse although it rapidly yielded to riboflavine in a few days Jolliffe s cases cleared up with daily doses of 5 mg [89]

Sydenstricker [86] and his colleagues have also described five patients who showed evidence of pellagra or were pellagrins and presented lesions corresponding to those described by Sebrell and Butler The cheilosis and fissures in the corners of the mouth disappeared when riboflavine was given in rather large doses of 20 to 75 mg a day orally or 10 to 50 mg parenterally the riboflavine seemed more effective parenterally than orally In every instance response to riboflavine was relatively slow and in the presence of an inadequate diet nicotinic acid given concurrently seemed to have no adjuvant effect Particular interest was aroused in two cases in which dermatitis cheilosis and conjunctivitis appeared to be cured by riboflavine

Although Kruse and Horwitt and co workers [91 260] were able to duplicate Sebrell and Butler s results some doubt has been cast upon the experimental production of pure riboflavine deficiency Thus Boehrer Stanford and Ryan [195] were unable to observe manifestations of riboflavine deficiency in volunteers on a daily riboflavine intake of 0 47 mg The experiment only lasted for five

before symptoms appear

reproduce the oral and

kept volunteers on diets

of ten months without observing any signs ascribed to riboflavine deficiency Clinical examination of the volunteers showed no abnormal findings and the following laboratory tests were normal serum calcium phosphorus and protein blood lipids blood counts blood glucose pyruvic acid gastro intestinal motility as shown radiologically urinalysis and slit lamp examination of the eyes Machella and McDonald [208] doubt the existence of the ariboflavinosis syndrome They have treated twenty patients with lesions attributed to the syndrome with riboflavine without success It is possible that not only riboflavine deficiency but deficiency of other factors of the vitamin B complex plays a part in producing the syndrome known as ariboflavinosis [240]

Validity of Angular Stomatitis * as a Manifestation of Riboflavine Deficiency Angular stomatitis is not a specific sign of ariboflavinosis and may occur

* This is referred to as cheilosis in much of the literature cited. It is preferable to retain cheilosis for the lesions of the vermilion of the lips

independently of the latter. Spies [107, 210] has shown that it commonly occurs in children and that *Staphylococcus aureus* and *Streptococcus hæmolyticus*

FIG 87 Ariboflavinosis. The lids, particularly the lower, of both eyes are macerated and stuck together. There are also long wide fissures at the angles of the mouth (angular stomatitis).

FIG. 88. Ariboflavinosis. The case shows dermatitis of both eyelids, extending from the margin of the lids to 3 to 6 mm. outwards with a dark red discoloration and papular lesions and crusts of exudates scattered over the lesion. The lesions responded to treatment with riboflavine and healed in six days.

can frequently be cultured from the fissures. The condition often deve in children who dribble or constantly lick because of the abnormal am of moisture at the corner of the mouth, whence the name perlèche, from *le*

to lick One of the authors has also observed fissuring at the corners of the mouth in two cases of Parkinsonism in which there was no question of nutritional deficiency and in which the condition was undoubtedly due to drooling of the saliva Ellenberg and Pollack [211] have observed deep granulomatous fissures at the corners of the mouth with no involvement of the lips and glossodynia in thirty four patients with no history of any nutritional defect and in whom laboratory studies showed no sign of avitaminosis There was no response to intensive riboflavine therapy either by mouth or parenterally The cause of the lesion was eventually traced to badly fitting dentures causing mal occlusion in thirty two of the patients and mechanical defects in closure of the jaws in the remaining two patients The skin at the corners of the mouth is constantly moist and becomes macerated and infected Other writers have also observed angular stomatitis due to badly fitting dentures and local trauma which did not respond to riboflavine but disappeared when the dentures were changed There is also evidence that angular stomatitis may be associated with such diverse conditions as hypochromic anæmia [213 214] sensitivity to the constituents of chewing gum [215] denture plastics [209] and the dye in lipstick [218] Other causes that have been incriminated are plastic cigarette holders some toothpastes antiseptic lozenges moustache wax cosmetics and the chewing of tobacco Angular stomatitis may also occur in chronic illnesses especially arthritis and as a result of treatment with antibiotics such as penicillin aureomycin and chloramphenicol [324] It is probable that angular stomatitis due to iron deficiency is more common than that due to riboflavine [213]

Numerous other reports show that the relationship of angular stomatitis to ariboflavinosis is not as clear cut as originally suggested and that as a symptom of the latter it is non specific Machella and McDonald [127 208] failed to improve thirteen cases of angular stomatitis by treatment with riboflavine Some responded however to nicotinic acid vitamin B_6 and the entire vitamin B complex given as brewers yeast Smith and Martin [216] also noted that angular stomatitis may disappear after the administration of vitamin B_6 The observation of Machella [127] that hæmorrhagic lip lesions may respond to ascorbic acid suggests that some cases are scorbutic Youmans [222] also found that some cases of angular stomatitis were refractory to treatment with riboflavine

Dermal Lesions of Ariboflavinosis In the experimental production of ariboflavinosis Sebrell and Butler [82 87] noted in addition to the labial changes a seborrhœic dermatitis of the face present on the alæ nasi naso labial folds eyelids and ears They were described in greater detail by Jolliffe [89] Spies [90] Sydenstricker [86] and others One of the first descriptions of this seborrhœic dermatitis in pellagrins was given by Stannus [84] The facial lesions consist of filiform excrescences of a seborrhœic nature apparently derived from sebaceous glands varying in length up to 1 mm closely and sparsely scattered over the face Although the characteristic location is in the nasolabial folds the excrescences occur on the alæ nasi the bridge of the nose above the eyebrows about the ears and other parts of the body (Fig 89) The lesion has the appearance of a seborrhœic dermatitis on an erythematous base the skin over the excrescences is fine

malar eminences and forehead Seborrhœic changes are stated to be present in the more severe cases of deficiency In many cases there is also a crusty superficially eroded lesion just inside the nares and there may be a vertical fissure at the mucocutaneous junction (Fig 89) The eyelids often show a dermatitis and may be macerated and stuck together [105] as shown in Figs 87 and 88

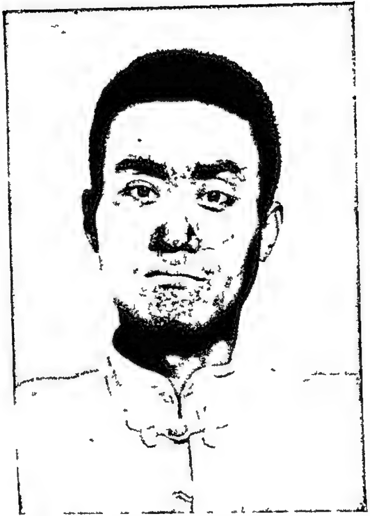


FIG 89 Ariboflavinosis before Treatment A pellagrin showing filiform seborrhœic excrescences on the fore head nose cheeks lips and chin and around the naso labial folds There are also moist pale patches in both corners of the mouth with vertical fissures on the lips

FIG 90 Ariboflavinosis after Treatment with Riboflavine The same patient as in Fig 89 after treatment with riboflavine (15 mg for the first two days 10 mg for the next seven to ten days and then 5 mg daily for another week) The lesions have disappeared

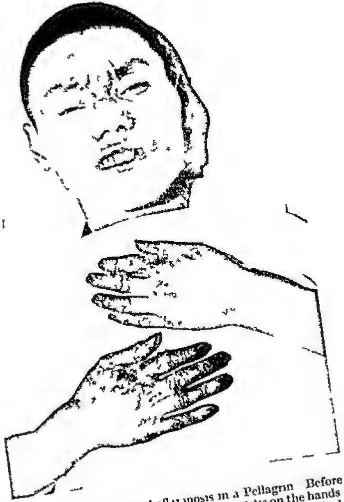


FIG 91 Lesions of Ariboflavinosis in a Pellagrin Before treatment There is a pellagrous dermatitis on the hands At the corners of the mouth are fissures around which serous crusts have accumulated The lips are dry and chipped and there are seborrheic excrescences in the naso labial folds both sides of the nose and the bridge and in between the eyebrows Photophobia is also present These lesions are characteristic of ariboflavinosis

FIG 92 Ariboflavinosis in a Pellagrin After treatment The same patient as in Fig 91 after treatment with riboflavine The lesions have practically disappeared

Sydenstricker [217] also mentions a dry brown itching dermatitis of the hands and scrotum or vulva associated with cheilosis and glossitis and responding to the riboflavine therapy. Purcell [143] describes a syndrome which includes scrotal dermatitis and superficial glossitis and which disappears on treatment with riboflavine. Mitra [219] gives an account of an oro-genital syndrome among Indians characterized by angular stomatitis, glossitis an scrotal dermatitis. The lesions did not respond to nicotinic acid but did t yeast or 5 mg. of riboflavine daily. Some cases, however, appeared to do better on a mixture of riboflavine and nicotinic acid. As far back as 1911 Stannus [84] described a dermatosis of the free border of the prepuce, the vulva, anus and scrotum, spreading to the thighs, and he now thinks that this is part of the riboflavine deficiency syndrome [240]. Scrotal dermatitis is one of the commonest skin lesions.

Tongue Lesions of Ariboflavinosis. Jolliffe [89], Kruse [91] and their co-workers have described a specific type of glossitis associated with riboflavine deficiency and quite distinct from that attributed to nicotinic acid deficiency. The tongue in the latter may be sore before any changes are visible. Then desquamation of the epithelium of the lingual papillae occurs, beginning at the tip and sides of the tongue and finally spreading over the entire dorsum. The tongue is scarlet, dry, atrophic and very painful. The tongue in ariboflavinosis on the other hand is described as magenta or purplish red in colour. It is clean and the epithelium does not desquamate over the papillae, but is flattened and swollen. The papillae are flattened or mushroom-shaped rather than atrophic, giving the tongue a pebbly or granular appearance. The tongue may be painful and burn when food is taken. Later irregular patchy denudation may occur giving rise to a condition known as "geographical tongue." Sydenstricker and his co-workers [115] have examined the papillae microscopically and liken them to "a dead jelly fish that has been washed up on the beach . . . a round translucent hemisphere with the capillaries lying deeply in a loose coil." The scarlet tongue of pellagra or nicotinic acid deficiency owes its colour to desquamation of the epithelial cells of the papillae; the capillary loops then become more readily visible. In the magenta tongue of ariboflavinosis the capillaries dilate and proliferate and possibly the circulation is slowed. This may account for the magenta colour of the tongue in contrast to the pink colour of the normal tongue.

Weisberger [220] describes a type of glossitis in riboflavine deficiency which does not correspond to the generally accepted picture. He states that the glossitis is characterized by a primary lingual coating followed by a patchy oval desquamation with an atrophic centre and raised edges. Weisberger states that this type of glossitis responds to treatment with riboflavine. Sydenstricker [115] noted a magenta coloured glossitis in pellagrins on diets poor in riboflavine treated with nicotinic acid. The tongue returned to normal on exhibiting riboflavine. The tongue changes are not reversed by riboflavine in long standing cases of deficiency.

Rosenblum and Jolliffe [221] record that some patients suffering from vitamin deficiencies still had glossitis even when treated with both nicotinic acid and riboflavine and that it did not return to normal until the whole vitamin B complex and vitamin B_6 were given. Machella and McDonald [208] failed to improve six patients with the so-called magenta tongue of ariboflavinosis by giving them riboflavine. Some did improve when yeast and vitamin B_6 were given.

Painful tongue (glossodynia) is not always due to riboflavine or other vitamin deficiency. It is often due to iron deficiency, excessive smoking, irritating foods and drink, electrogalvanic currents produced by dissimilar metallic dentures and neurogenic and psychogenic causes [226]. It may be the only symptom of thrombosis of a small intracranial vessel (Alvarez). Smith [37] describes a number of cases of fissured tongue with changes in the epithelium and papillae that did not respond to treatment with riboflavine.

and other B vitamins Glossitis may also occur in pernicious anæmia sprue and pellagra and as a complication of oral treatment with antibiotics such as chloramphenicol and aureomycin [324]

Ocular Manifestations of Ariboflavinosis In 1939 Spies and his co workers [90 228] observed in patients suffering from malnutrition an ocular lesion characterized by bulbar conjunctivitis lacrimation burning of the eyes and failing vision that was cured by administering riboflavine At the same time Sydenstricker and his colleagues [86] in a study on riboflavine deficiency noted that conjunctivitis and photophobia were prominent symptoms In the same year Pock Steen [45] described twilight blindness in patients with sprue or incipient sprue that was relieved by riboflavine and not by vitamin A Many of these patients also suffered from ocular conditions such as reduced visual acuity conjunctivitis keratitis and mydriasis which were attributed to riboflavine deficiency The work of Bessey and Wolbach [41] and Eckhardt and Johnson [67] in 1939 showed that the earliest sign of riboflavine deficiency in the rat is corneal vascularization This was followed

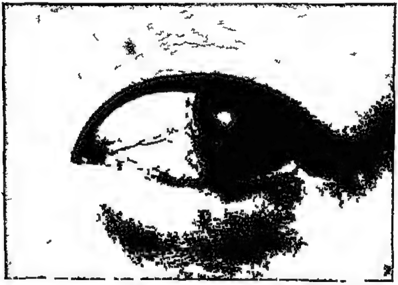


FIG 93 Ariboflavinosis A case showing injected vessels in the conjunctivæ corneal opacities and phlyctenules at the margin of the cornea

in 1940 by the papers of Kruse Sydenstricker and their colleagues [91 92 115] on the ocular changes of riboflavine deficiency in man They stated that corneal vascularization was a constant finding It is now known that this is not pathognomonic of riboflavine deficiency although it may occur in cases of ariboflavinosis (p 317)

Conjunctivitis Gross injection of the vessels of the bulbar and fornix conjunctivæ have been described in subjects with riboflavine deficiency [91–93] This has been referred to as conjunctivitis although no infection was present (Fig 93) Hou [105] who has seen many cases in China adds phlyctenular conjunctivitis as a symptom of ariboflavinosis He states that in China the ocular lesions are more commonly seen than the facial and oral ones

It is stated that in India many patients with angular stomatitis and other signs of ariboflavinosis also suffer from angular conjunctivitis of the Morax Axenfeld type [293] This conjunctivitis as well as the other signs of

treatment with 3 to 5 mg

ng daily were needed

as observed in forty three

out of forty seven patients with ariboflavinosis examined by Sydenstricker [92]

Johnson and Eckhardt [93] and Hou [105] also found that it was a promi[illegible] symptom in their patients. Itching, burning, blepharospasm, a sensatio[illegible] roughness of the eyelids, lacrimation, mydriasis, blurred vision, inabilit[illegible] see in a dim light and visual fatigue are also described as common in pati[illegible] with ariboflavinosis [91, 92, 115]. These lesions clear up in twenty-four forty-eight hours after giving riboflavine.

Diminished Visual Acuity and Eye Strain. Diminished visual acuity w[illegible] observed in twenty-nine of forty-seven patients studied by Sydenstricker [9[illegible] It was also noted in subjects with ariboflavinosis by Johnson and Eckhar[illegible] [93] and by Hou [105]. The symptoms occurred in the absence of errors [illegible] refraction or opacity of the lens. Impaired visual acuity may result fro[illegible] such symptoms as burning of the eyes, lacrimation, blepharospasm, corne[illegible] opacities and iritis, which are all stated to occur in ariboflavinosis. Vitamin [illegible] deficiency may cause some of these symptoms, and it is quite likely that a diet deficient in riboflavine—that is, lacking an adequate quantity of milk, eggs, green vegetables and whole grain—is also deficient in vitamin A. The work of Kimble and Gordon [44] suggests that riboflavine may be necessary for the proper utilization of vitamin A. They found that some subjects with poor dark adaptation did not respond to treatment with vitamin A unless riboflavine was given as well. It is possible that the diminished visual acuity, photophobia and "twilight blindness" considered to be associated with riboflavine deficiency may be in part due to interference with the regeneration of visual purple in the rods and cones. Sydenstricker and others [92] report considerable improvement in visual acuity in their cases treated with riboflavine.

Eye strain has been stated to fall into this group. Pett [229] states that of a group of 232 persons doing close work and with many complaining of eye strain, thirty-seven per cent showed signs of riboflavine deficiency. Twenty-eight were given 3 mg. of riboflavine daily for three months and sixteen showed much improvement. However, a similar number in a control group showed improvement on a placebo. This illustrates the importance of suitable controls in studies of this kind.

Corneal Opacities. Bessey and Wol[illegible] deficiency in rats noted opaque in[illegible]ge of the limbus, and Sydenstricker a[illegible]orkers [92] observed corneal opacities in eighteen of his forty-seven cases of ariboflavinosis. Superficial nebulae seen by the naked eye as a slight "steaminess", and on slit lamp examination as a fine superficial diffuse opacity, were noted in all these cases and superficial punctate opacities in two of them. Interstitial nebulae and posterior punctate opacities were sometimes seen, although rarely. Johnson [130] noted corneal ulcers appear over these opaque infiltrates in severe cases of ariboflavinosis. These corneal opacities are probably caused by infiltration of the corneal epithelium and substantia propria with leucocytes. With riboflavine therapy these corneal nebulae and ulcers heal, often completely, but if untreated the opacities become permanent and scar tissue may form from corneal ulcers. According to Sydenstricker [92] the interstitial nebulae clear more rapidly than the superficial ones, and the posterior nebulae disappear last of all. It must not be forgotten that a deficiency of other vitamins may result in corneal lesions. Thus vitamin A deficiency may cause corneal opacity, scarring of the cornea and even perforation in severe cases (p. 73), and corneal lesions are also seen in animals on diets deficient in pantothenic acid, tryptophane, pyridoxine and the amino acids isoleucine and valine [224, 255].

A deficiency of vitamin A and riboflavine may in fact occur in the same individual. Verma [232] describes a syndrome which appears to combine the manifestations of a deficiency of both these vitamins and which is characterized by partial degeneration of the optic nerve, phrynoderma, sore mouth, night blindness, xerosis, conjunctivitis, photophobia and impairment

of vision. The condition responds to treatment with shark liver oil, a potent source of vitamin A, and yeast, a source of the vitamin B complex including riboflavine. The oral lesions cleared up with riboflavine alone

Spies and his co-workers [42] observed corneal ulceration and infected exudates in riboflavine deficient subjects and state that ocular symptoms may occur independently of cheilosis and other symptoms

Cataract. Conjunctivitis and keratitis occur in animals on diets free from riboflavine, followed by dullness of the eyeball and finally, according to some observers, opacity of the lens, although the latter observation has been doubted (p 296) In Sydenstricker's series [92] cataract was observed in six, but they were all elderly patients and it is difficult to say whether the cataract was the result of riboflavine deficiency. According to Wagner, Richner and Karbacher [68] riboflavine does not arrest the progress of cataract in the human eye. A deficiency of other factors may cause cataract in the experimental animal, e g. tryptophane [230] In man it cannot be considered as yet that cataract is a result of ariboflavinosis

Iritis. Sydenstricker and his co-workers [91, 92] observed severe iritis in four out of forty-seven of their cases, and mild iritis, characterized by moderate congestion of the iris with accumulation of pigment on its anterior surface, in somewhat under half of the cases. In light-coloured irises the pigment appeared as dark clumps of "hazel spots", in brown irises the *pigment caused "veiling of the normal architecture."* As *these changes* disappeared with riboflavine therapy they were considered to be part of the ariboflavinosis syndrome

Rubeosis Iridis. This is a peculiar non inflammatory vascular proliferation affecting the iris and mostly seen in diabetics, although diabetes is not an essential factor in its causation Festoons of newly formed blood vessels are seen on the surface of the iris, and in some cases the condition appears more particularly in the sphincter region, where the vessels anastomose to form a network encircling the pupil In other cases patches of anastomosing vessels are seen in the periphery Stannus [233] suggests that rubeosis is a manifestation of riboflavine deficiency, as he has cured the condition with riboflavine in doses of 5 mg daily After forty eight hours the vascular network on the iris is difficult to see, and after a week completely disappears

Nutritional Amblyopia. Métivier [128] has given an account of ocular *manifestations observed in Trinidad which he considers are due to riboflavine* deficiency One he calls "tropical nutritional amblyopia," which is the *nutritional "retrobulbar neuritis" of Moore [246, 247]*, Stannus [84], *Landor* [illegible] "malnutritional amblyopia" [illegible]. Many of the earlier writers [illegible]e aware of some of these eye [illegible] acuity, diminution in the size of the visual fields dimness of vision, flickering of images, disappearance of images, difficulty in recognizing objects and persons, rings and haloes about lights at night, disturbances of colour vision, scotomata, pallor of the temporal halves of the discs, and partial optic atrophy. Some patients describe how objects come into vision and then disappear ; others say they can only see parts of printed words. These symptoms are attributed to failure of the nutrition of the optic nerve or the retinal elements and according *to more recent writers, are the result of ariboflavinosis, since recovery from* some of the symptoms occurs on administration of 4 to 5 mg of riboflavine daily [128] Foods rich in the vitamin B complex, such as wheat germ, yeast and marmite, are also effective in causing improvement Wilkinson and King [256, 301] have described a deficiency syndrome seen in Hong Kong in 1940 with amblyopia as a predominant symptom accompanied by soreness of the tongue, angular stomatitis, giddiness, weakness of the limbs, temporal pallor of the discs, acroparæsthesiæ, scrotal eczema and swelling of the ankles Visual acuity was reduced in some cases to finger counting at 3 feet within a

few weeks of onset The condition cleared up with yeast and dietetic measures and also with nicotinic acid and riboflavine, although the authors regarded the syndrome as a result of nicotinic acid deficiency. The amblyopia, however, cleared most rapidly when the patients were given riboflavine. Thus 3 mg of riboflavine daily brought vision from 6/60 to 6/9 or 6/6 in a week or ten days It was also found that a full well-balanced diet helped to restore visual acuity The complete syndrome is more likely to be due to a deficiency of riboflavine and protein rather than of nicotinic acid.

Métivier [128] claims that he has observed 192 cases of a condition hitherto unrecorded which he calls essential corneal epithelial dystrophy It is characterized by a faint greyish-white disturbance in the corneal epithelium made up of fine points like dots and commas, and it runs typically in a double line transversely across the cornea at the level of the lower part of the pupillary areas It stains with fluorescein or Bengal red At times the double line is incomplete, but minute prolongations usually extend above and below it, in some cases almost the whole of the corneal surface is covered with faint greyish-white points Other symptoms associated with this eye condition are photophobia, lacrimation, pallor or atrophy of the temporal half of the optic disc, burning and numbness of the feet, cheilosis, dry parched skin, sore tongue and "rosy eyes" The last-mentioned is an apple-pink injection of the ocular conjunctiva exposed in the interpalpebral area with dilated vessels that run into the limbus Recovery occurs from the epithelial dystrophy and rosy eyes after two weeks' treatment with 5 mg. of riboflavine daily, and only after five to twelve weeks when foods rich in the vitamin B complex are given The burning and numbness of the feet were only relieved by aneurine

A number of papers were written in the immediate post-war period on nutritional amblyopia seen in prisoners of war, mainly those in Japanese hands The observations were carried out by physicians many of whom had never left their homeland before, and, deprived as they were of access to the literature, most of them believed they were describing a new syndrome They added little to the clinical descriptions of the earlier workers on the subject; being prisoners of war themselves and living under terrible conditions, it is remarkable that they kept such good clinical records Among the authors are Spillane and Scott [78], *Talbot* [79], *Smith* [88], *Reed* [212] Smitskamp [252], Wilcockson [239] and De Raadt [259] De Raadt describes Pick's visions, or partial visual vertigo, in which some part of the visual field moves in relation to the surrounding objects, e g letters may appear to be detached *from a word or a person* may appear to move into an adjoining room through a wall

Snow-blindness According to Tisdall [237] snow blindness may be due to riboflavine deficiency He examined 400
that five per cent were blind. The blindi
deficiency because the diet is poor in ril
reflected from the snow, being very strong, causes local destruction of riboflavine in the eye Pure riboflavine is destroyed by ultra-violet light, but in the eye it is present as riboflavine-adenine dinucleotide, which is not inactivated by *light* [250]

Corneal Vascularization. In 1940 Sydenstricker, Sebrell, Cleckley and Kruse [91, 92] examined the eyes of forty seven patients with riboflavine deficiency and noted vascular changes in forty five of them They stated that the earliest and most common sign of ariboflavinosis is circumcorneal injection (Fig 95), that is, proliferation and engorgement of the bulbar conjunctival capillaries of the limbic plexus The lesion, if not grossly visible, is seen on slit lamp examination They describe the earliest change as marked proliferation and engorgement of the limbic plexus with the production of great numbers of narrow capillary *loops*, which *outline* the extreme margins of the scleral digitations and obliterate the narrow avascular zone

between the plexus and the sclerocorneal junction They state that if untreated the lesion progresses to corneal vascularization within a short time but rapidly regresses if treated with riboflavine In untreated cases the cornea is described as being invaded first by very small capillaries arising from apices of loops surrounding the scleral digitations and lying just beneath the epithelium They soon anastomose to form a tier of loops from which more capillaries develop and extend centripetally to form secondary arcade capillary loops The process of anastomosis and loop formation proceeds until extensive vascularization of the cornea results (Fig 95) The capillaries are empty at first but fill with red blood cells in a few days Many of the vessels are so small that they are invisible to the naked eye or with a loupe but are visible under the corneal microscope or slit lamp The Sydenstricker school believe that these eye lesions are due to riboflavine deficiency because they clear up when riboflavine is administered they are present in persons living on diets poor in riboflavine the administration of other vitamins has no effect the lesions are associated with cheilosis and glossitis and similar lesions are produced in animals kept on diets deficient in riboflavine

Bessey and Wolbach [41] from animal observations state that corneal vascularization is an early and specific sign of riboflavine deficiency and occurs as a compensatory mechanism to bring the blood into closer contact with the corneal epithelium This being normally avascular contains no hæmin compounds and Wolbach and Bessey assume that probably the

proximity with the corneal cells This view has been widely accepted The work of Philpot and Pirie [250] however makes this explanation unlikely They have shown that whereas the cornea contains less riboflavine than any other ocular tissue (0 2 microgram per gram) the lacrymal gland contains as much as 6 5 micrograms per gram They therefore suggest that the cornea receives its riboflavine from the lacrymal secretions rather than from the blood of the limbic plexus

Validity of Circumcorneal Injection and Corneal Vascularization as Manifestations of Riboflavine Deficiency After the observations of Sydenstricker and his colleagues in 1940 on the eye symptoms of ariboflavinosis circumcorneal injection and corneal vascularization were accepted as certain diagnostic signs of the condition and were used in nutrition surveys as an index of riboflavine nutrition Since 1942 however many investigations have shown that neither of these signs is diagnostic of riboflavine deficiency although they ma

Stannus [240]

eyes by slit lamp

not a narrow avascular zone between the limbic plexus and the corneo scleral junction and that he has seen both circumcorneal injection and corneal vascularization without any evidence of riboflavine deficiency He states that the area between the limbic plexus and the corneo scleral junction may appear avascular because the blood vessels may be constricted and empty They become visible however on using drugs such as dionine which dilate the vessels The limbic plexus which is a capillary bed and liable to great variation within physiological limits becomes congested and engorged on the slightest provocation Engorgement occurs in all varieties of conjunctivitis in those whose eyes are exposed to heat and dust cold wind bright light mild infection chemical irritants and even by rubbing the eye [241 242] The proliferation of the limbic vessels is thus only apparent because vessels which were once empty and invisible become filled with blood and visible Hence circumcorneal injection is not pathognomonic of riboflavine deficiency

The work of Scott [257] Ferguson [242] Gregory [241] Stannus [232 240] and others has conclusively shown that corneal vascularization can occur in the absence of riboflavine deficiency Anderson and Milam [77] in a nutritional survey of over one thousand individuals both black and white found

Fig 94 Normal Eye There is no proliferation of the limbic vessels and no penetration of cornea by blood vessel

associated with photophobia impaired vision itching a[illegible] grittiness in the eyes The limbic plexus is congested and there is a bilateral symmetrical superficial *vascularization of the cornea* in which small vessels grow inwards from the marginal loops of the *limbic* plexus under the *corneal*

epithelium in a regular arcade all round the cornea and extend towards the centre. According to Stern [311] riboflavine deficiency always causes corneal vascularization if it is continued long enough. He believes that the condition may be precipitated by conditioning factors such as chemical or mechanical

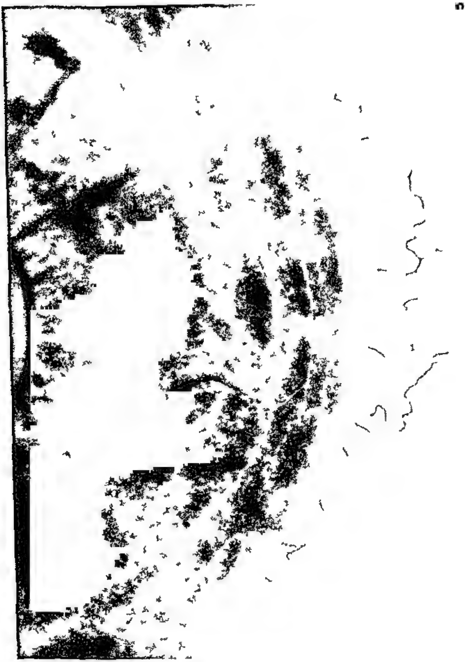


Fig. 95. An eye showing circumcorneal injection. This was claimed by Sydenstricker to be an early sign of ariboflavinosis. It is not pathognomonic.

trauma to the cornea in the presence of a subliminal riboflavine deficiency.

Some other conditions in which corneal vascularization may occur are vitamin A deficiency [41], tryptophane deficiency [230, 255], injury to the corneal epithelium by chemical irritants, diseases causing pannus such as trachoma, phlyctenular keratitis and any superficial keratitis [241]. The instillation of a simple irritant such as five per cent. soap solution into the

flavine It is unlikely that kwashiorkor results from a pure riboflavine deficiency, inanition protein deficiency and dehydration probably play a part

Pathogenesis of Ariboflavinosis Stannus [240] believes that the varied lesions of riboflavine deficiency are a manifestation of an acute functional derangement of the capillary circulation of the affected parts The first tissue to suffer from riboflavine deficiency is the endothelium of the capillary system The capillaries undergo a reversible functional disturbance—a *capillary dysergia—resulting in loss of tone and dilatation* Normal cellular metabolism is upset and tissue functions are disturbed The first tissues to

of capillaries those whose metabolism
alized function The interference with
e nature of an anoxia using this term

in its widest sense If the anoxia is not too prolonged, recovery of function takes place in the capillaries when supplied with adequate riboflavine otherwise irreversible processes leading to pathological changes occur According to Stannus the skin lesions of ariboflavinosis which occur where the skin is thin or highly specialized and at the mucocutaneous junctions about body orifices (lips, palpebral fissures nares prepuce vulva anus

aired nutrition of
t esult of capillary
(that the changes
i he believes that
t the eye, but are

part of a lesion in the central nervous system The neurological manifestations are the expression of a metabolic disturbance in nerve tissue produced by capillary dysergia the more vascular tissues being the first to suffer e g the grey matter and the cerebellar neurophyl Stannus [253 292] points out that the neurological lesions of ariboflavinosis are essentially affections of the sensory nerves

Diagnosis of Ariboflavinosis The diagnosis of ariboflavinosis is made on the history, clinical examination and response to treatment with riboflavine If the condition does not rapidly respond to treatment with riboflavine it is not ariboflavinosis Laboratory tests have proved disappointing Blood and urine studies have been made and saturation tests have been devised for the laboratory diagnosis of riboflavine deficiency, but they are of doubtful value and have not received general acceptance as normal standards have not been laid down (p 300) Suvarnakich Mann and Sure [335] however believe that the riboflavine content of serum should be of value in the diagnosis of human riboflavine deficiency They state that the serum level of free riboflavine and flavine adenine dinucleotide in normal subjects is 0 84 $\pm$ 0 71 micrograms and 2 32 $\pm$ 0 42 micrograms respectively per 100 ml Nitrogen balance and protein intake do not affect these levels It is generally accepted that ariboflavinosis cannot be determined by single estimations of the twenty four hour excretion of riboflavine [126] It is also possible to depress the excretion of riboflavine to zero without producing evidence of deficiency symptoms [126] Feder Lewis and Alden [302] state that an excretion of 0 53 to 0 8 microgram of riboflavine per ml of urine denotes an adequate intake while values below 0 3 microgram per ml denote a deficient intake of riboflavine According to Johnson and his co workers [314] excretion while fasting of less than 20 micrograms per hour is the lower limit of normal and 200 micrograms the lower limit four hours after an oral test dose of 5 mg Sinclair [315] gives an excretion of 30 micrograms per hour while fasting as abnormal and 10 micrograms as denoting deficiency, he states that a blood level of less than 12 micrograms per 100 ml also denotes deficiency Lossy Goldsmith and Sarett [329] however, have shown that riboflavine excretion is not diminished in patients suffering from clinical vitamin B complex deficiency

In the clinical examination the characteristic seborrhoeic facial lesions the

glossitis, and the lesions in the region of the anus, vulva and prepuce are of diagnostic importance, but circumcorneal injection and corneal vascularization, although they may be present, are non-specific (p. 317). Angular stomatitis may be present, but it may be due to causes other than ariboflavinosis (p. 309). The neurological lesions of diagnostic value are burning feet, retrobulbar neuritis followed by partial optic atrophy, loss of visual acuity, sensory changes, and the cerebellar syndrome of Stannus (p. 320). A poor dietary history—one lacking meat, cheese, eggs, green vegetables, milk—is suggestive. Diagnosis may be assisted by the therapeutic test. If the lesions do not show some signs of resolution in a few days after giving 10 mg of riboflavine daily, the condition is not due to riboflavine deficiency.

In the differential diagnosis of ariboflavinosis, pellagra, sprue, idiopathic hypochromic anæmia, subacute combined degeneration, disseminated sclerosis, and cerebellar lesions must be considered. Glossitis, lesions of the lips, anus, scrotum and vulva, burning feet, muscular weakness and retrobulbar neuritis may occur in pellagra. Angular stomatitis and skin lesions similar to those seen in ariboflavinosis have been reported in sprue and idiopathic hypochromic anæmia [103]. As Landor and Pallister [248] pointed out, the neurological symptoms in the late stages of what is now known as ariboflavinosis resemble those of subacute combined degeneration (paræsthesiæ of legs, ataxia, impaired appreciation of touch, pain and temperature, rombergism, exaggerated knee and ankle jerks, nystagmus, achlorhydria, glossitis). Disseminated sclerosis may also be confused with some of the neurological symptoms reported in ariboflavinosis (retrobulbar neuritis, misty vision, paræsthesiæ, muscle weakness, nystagmus, vertigo, emotional changes, exaggerated reflexes). The neurological manifestations listed by Stannus (p. 320) are almost identical with those of the cerebellar syndrome.

Treatment of Ariboflavinosis. Most cases respond to treatment with riboflavine in doses of 5 to 10 mg. by mouth daily. The average case responds to 5 mg. by mouth daily [115], although Jolliffe [89] prefers to give 50 mg. intramuscularly at the beginning of treatment for a few days, followed by 10 mg. daily by mouth. If the patient suffers from achlorhydria, vomiting, diarrhœa, hepatic disease or other disorder preventing absorption or utilization, the riboflavine is given parenterally in doses of 10 mg. of the sodium compound [92, 115]. Larger doses, although wasteful, are non-toxic [81]. Yeast in doses of 60 to 90 grams daily is also curative. Sydenstricker [86] warns against giving large doses of a single vitamin for long periods in the treatment of avitaminosis, as although it may cure the major manifestations of the condition under treatment, it may precipitate a deficiency of another member of the vitamin B complex (p. 240). He therefore gives other members of the B complex such as yeast, crude liver extract, wheat or rice bran extract with the riboflavine. Food yeast (*Torulopsis utilis*) is a rich source of riboflavine, and 10 grams a day is sufficient to eliminate riboflavine deficiency at a cost of one farthing a day [8]. In addition to vitamin therapy the diet must include generous amounts of food rich in the vitamin B complex, e.g. whole grain cereals, meat, milk, liver, eggs and cheese, otherwise the patient will relapse as soon as riboflavine is withdrawn.

Following the administration of riboflavine, photophobia, burning, itching and blepharospasm are relieved in twenty-four to forty-eight hours and visual acuity slowly improves. Oral lesions begin to improve in three days or so, but complete resolution may take weeks [91, 92].

The improvement in the glossitis can be followed by tongue prints. The tongue is wiped dry and covered with ink by means of an inking pad. Then stiff white glossy paper is placed on the tongue with a rolling motion and quickly removed. Serial tongue prints so obtained are valuable in following the type and progress of the glossitis (Figs. 111 to 120).

There is no information on the speed with which the neurological manifestations attributed to ariboflavinosis resolve.

Plummer-Vinson's Syndrome This syndrome is characterized by glossitis anæmia dysphagia and achlorhydria and is practically confined to women It has been ascribed to ariboflavinosis [112 225] on account of some resemblance in the symptomatology of the two conditions although there are conflicting reports on the value of riboflavine in the treatment of the

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riboflavine deficiency The increased sedimentation rate and the frequent involvement of joints suggests however an infective ætiology

Clinical Uses of Riboflavine

Apart from its use in the treatment of deficiency states the value of riboflavine in therapeutics has yet to be shown

Johnson and Eckhardt [93] noted the resemblance between acne rosacea and the flushing of the face and prominence of capillary dilatation on the cheeks and nose of some of their patients with ariboflavinosis (They therefore suggested that acne rosacea is due to riboflavine deficiency the persistent

ectases seborrhoeic hyperactivity with dilated

hyperactivity of the subcutaneous tissue being

nse of the blood vessels to deficient oxidative

of capillary dysergia p 321). Johnson and Eckhardt claim that the condition responds to treatment with 1 to 2 mg of riboflavine daily although Sulzberger and Cope [313] were unable to confirm this Johnson and Eckhardt further state that they isolated *Demodex folliculoris* from the skin of rosacea patients and that they were able to infect with it the skin of riboflavine deficient rats but not the skin of normal rats They believe that the *Demodex* is a secondary invader of skin containing dilated capillaries

Johnson and Eckhardt [93 130 192] state that rosacea keratitis is of nutritional origin They noted that the diet of patients with this condition is low in milk liver and eggs—good sources of riboflavine—and that many of them show some degree of riboflavine deficiency as judged by retention tests Patients with rosacea retained 47 5 per cent of a dose of 5 mg given intramuscularly compared with normal controls who retained 21 5 per cent The validity of these tests is open to doubt Conners Eckhardt and Johnson [192] postulate that corneal disease may result from a dietary deficiency of riboflavine or from a disturbance of riboflavine metabolism They claim to have successfully treated rosacea keratitis marginal corneal ulcers and catarrhal corneal infiltrates with 1 mg riboflavine intravenously daily supplemented by a vitamin B complex preparation containing 830 micrograms of riboflavine per ounce 1 ounce being taken orally three times a day

There is a suggestion that psoriasis improves if patients are treated with riboflavine intramuscularly (5 to 10 mgm) [336]

As Wise [270] points out many factors including riboflavine deficiency which have been suggested as ætiological in rosacea keratitis are probably not fundamental although they may be contributory He administered large doses of riboflavine to twenty one patients with the condition but failed to cure the eye lesions Spontaneous remissions often occurred without any change in diet Fish [271 298] states that rosacea keratitis may occur without riboflavine deficiency and is not cured by treatment with it A series of forty five cases all of whom had cutaneous lesions as well failed to benefit from riboflavine therapy Many of them became worse owing to the withdrawal of atropine during the test Fish considers that neither acne rosacea nor rosacea keratitis are manifestations of ariboflavinosis and that most symptoms are due to secondary infection which can be controlled by the sulpha drugs She points out that the type of corneal vascularization seen in ariboflavinosis is not the same as that present in rosacea keratitis

Grimsdale [95] has used riboflavine with apparently beneficial results in the treatment of corneal ulcers, photophobia and non-infective conjunctivitis without any typical signs of ariboflavinosis. Rones and McKay [131] gave riboflavine to twelve patients with diverse chronic corneal lesions and without signs of riboflavine deficiency. The cases, which included phlyctenular keratoconjunctivitis, corneal ulceration, superficial punctate keratitis and sclerosing keratitis were all stated to benefit from the oral administration of 5 to 10 mg. of riboflavine daily, including cases which had not responded to previous local therapy. Stern and Landau [121] claim to have effectively treated several cases of eczematous keratitis with riboflavine; they consider that the condition results from a number of factors (infectious agents, mechanical irritation) associated with ariboflavinosis superimposed on a tuberculous diathesis.

Sydenstricker and his colleagues [91, 115] have observed several cases of syphilitic keratitis in which treatment with riboflavine was followed by rapid improvement during periods in which antisyphilitic treatment was not given. This has been confirmed by Cosgrove and Day [137] and Clark [140], although Wagener [100] failed to obtain any improvement. Kruse and Sydenstricker [91, 92] discuss the relationship of syphilis to ariboflavinosis and keratitis, and raise the question whether syphilis produces keratitis only when the nutrition of the cornea is impaired, as in ariboflavinosis.

Castellanos [276], accepting the erroneous assumption that strong light inactivates the riboflavine of the eye (p. 297), states that vernal conjunctivitis is caused by ariboflavinosis. Of one hundred and five patients treated with local anæsthetics, adrenaline and riboflavine, 1 to 3 mg. daily, he claims that ninety-two per cent. showed immediate improvement. Stern [106] confirmed this in the palpebral form with papillary hypertrophy, which improved under treatment with 5 mg. of riboflavine twice daily. As in neither case were the observations controlled and vernal conjunctivitis is a persistently recurrent condition, further proof is required of any association between it and ariboflavinosis.

Verma [293] observed that riboflavine is effective in the treatment of angular conjunctivitis of the Morax-Axenfeld type without giving any local treatment. Most of his patients were, however, suffering from symptoms of ariboflavinosis and the clearing of the conjunctivitis may well have been due to improvement in their general condition. Landau and Stern [118] noted improvement in patients with trachomatous pannus treated with riboflavine; these were also patients with symptoms of ariboflavinosis. Schwartzman, Dragutsky and Rook [97] reported the successful treatment with riboflavine of a case of Ritter's disease (dermatitis exfoliative infantum), the essential

in paraffin. Rapid improvement and cure in eighteen days were reported. Topping and Knoefel [99], on a purely experimental basis, claim to have treated a case of pemphigus with riboflavine after all other treatment failed but Wolf and Lewis [76] failed to observe any response in a case treated with 100 mg. daily. Vorhaus and his co-workers [272] report complete healing in five cases out of six of decubital ulceration treated with 5 mg. of riboflavine daily. Complete healing occurred in from seven to thirty four days without any other treatment.

It has been claimed that riboflavine and the vitamin B complex ... the oral side-effects sometimes seen after the administration of the antibiotics

chloramphenicol and aureomycin (atrophic glossitis, redness of the oral ... antibiotics alter the ... the development of ... with riboflavine or other vitamins can influence this as the symptoms although they superficially resemble those of ariboflavinosis, have a totally different pathology

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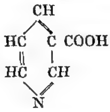
CHAPTER V

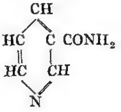
NICOTINIC ACID

NIACIN

HISTORY

NICOTINIC acid a member of the B complex is β pyridine carboxylic acid The amide nicotinamide is a component of a complex enzyme system


Nicotinic acid


Nicotinamide

For several acid in nutrition remained undiscovered and found in yeast concen trates nearly fc taken in the physiological role of pyridine rburg [1] and his colleagues showed that nicotinamide is the active group of the co enzyme now known as codehydrogenase II (p 339) At the same time Kuhn and Vetter [2] isolated nicotinamide from heart muscle and others obtained it from cozymase As a result of this work considerable interest centred around nicotinic acid as a factor in nutrition Thus Frost and Elvehjem [3] observed that it had growth stimulating properties and others showed that it was an essential growth factor for a number of micro organisms

A disease of dogs known as black tongue—characterized by glossitis stomatitis diarrhœa and typical skin lesions—bears a close resemblance to human pellagra During their studies on chick pellagra Elvehjem [4] and his associates isolated nicotinic acid from liver concentrates that were active in curing the condition They then tested the effect of nicotinic acid on dogs suffering from black tongue it was curative This suggested its therapeutic use in the treatment of human pellagra The first report of its successful use in this connection was made by Elvehjem Madden Strong and Woolley [4] in September 1937 A number of papers by other investi gators confirming this appeared in rapid succession Nicotinic acid was at first hailed as the PP or pellagra preventing factor but it is now known that pellagra is a multiple deficiency disease and that lack of nicotinic acid is only one of the factors in its causation

In 1942 the Food and Nutrition Board of the U S A National Research Council suggested the terms niacin and niacin amide for nicotinic acid and nicotinic acid amide respectively [156] The Food and Nutrition Board suggest that the original names be retained in scientific literature

CHEMISTRY OF NICOTINIC ACID

Nicotinic acid is a white crystalline solid melting at 228° 229° C soluble in water (1 in 60 at 25° C) and alcohol (1 in 80 at 25° C) It is not oxidized or destroyed in the ordinary processes of cooking or by exposure to air light or alkalis It can be made bacteriologically sterile by autoclaving

without loss of potency Being an acid it readily forms salts and an amide which are also physiologically active The pH of a 1 per cent solution is 3

Nicotinic acid has been estimated in body fluids and foodstuffs by a variety of methods [135] One chemical method depends on the colour produced with cyanogen bromide which can be estimated photometrically ... after condensation with various amines, such as aniline [8] ...

bromide can be estimated photo... spectrophotometry [14] Another method depends on the formation of a coloration when the ... material under test is fused with 2 4 dinitrochloro benzene [13] None ... by other pyridine ... [000] ... lacto bacillus test [12] in which the amount of lactic acid produced by *Lactobacillus arabinosus* is proportional to the nicotinic acid content of the medium Other methods depend on the use of bacterial enzymes present in bacteria which have been adapted to grow on nicotinic acid [155] and the use of *Proteus* HX19 [6] and yeast [7] A micromanometric method for estimating nicotinic acid combined as diphosphopyridine nucleotide (*codehydrogenase* I p 339) has been devised It depends on the liberation and measurement of carbon dioxide during a reaction catalysed by diphosphopyridine nucleotide The pyridine nucleotides can also be estimated ... on active charcoal elution with ten per cent pyridine and ... [019]

FIG 96 Nicotinic Acid Crystals

As a considerable ... erial is combined it is freed by vigorous hydrolysis ... This produces dark pigments that interfere with colorimetric estimation Either the pigment or the nicotinic acid is adsorbed or the pigment removed by extraction with non aqueous solvents such as ethyl acetate or ethyl laurate

DISTRIBUTION IN FOODSTUFFS

Nicotinic acid occurs in all living cells Liver the adrenals kidney yeast whole grain products flesh foods ('meat') mushrooms, and peanuts are among the best sources Denatured cereals such as white flour or polished rice fruit and vegetables in general and milk are poor sources Meat extracts contain appreciable quantities e g a teaspoonful such as is used to prepare a drink may contain 10 mg [164] Free nicotinic acid is not found in living cells but as the amide or as part of complex enzyme systems in which it is chemically bound (p 339)

Nicotinic acid is concentrated mainly in the aleurone layer of cereals and

the bran and not, like aneurine, in the scutellum In America bread is "fortified" by the addition of nicotinic acid so that the content is about 16 mg per pound It is recommended that in Britain bread should contain 1 6 mg of nicotinic acid per 100 grams of bread as a minimum [34] An increase in the nicotinic acid content of oats and rice occurs on germination, but not of wheat, barley or maize [35]

Since nicotinic acid is heat stable and resistant to oxidation and the action of light, very little is lost in food during cooking and processing Any losses that do occur result from the vitamin diffusing into the cooking water, which is usually thrown away In the cooking of meat eighty to eighty five per cent is retained after roasting, seventy seven per cent after frying and sixty five per cent after braising, the total retention in the meat and drippings in domestic cooking averages seventy per cent and may be as high as 100 per cent in stewing [19 165, 166] In the curing of meat some eighty-four per cent is retained [166] Food cooked in cafeterias and restaurants usually contains less nicotinic acid than food cooked at home Losses up to sixty per cent have been recorded [167] In the cooking of vegetables the loss of nicotinic acid is from eight to twenty two per cent, the cooking water contains twelve per cent on an average (range two to forty per cent) [170] In the dehydration and canning of meat products eighty eight to ninety four per cent of the nicotinic acid is retained [263], in the case of vegetables it is seventy seven to a hundred per cent [39] If the cooking water is thrown away it will be less

Nicotinic Acid Content of Foods

Food	Description	Nicotinic Acid in micrograms per gram
Cereal Foods		
Barley	Red	47
	Pearl	27 5–30
Bread, wheat	White	6 6 10 0
	Wholemeal	37–42
	National bread (1943)	10 6
	Enriched (U S A)	33
Biscuits (dry)	—	20–30
Buckwheat	—	44
Corn	—	15 6–26 0
	Meal	17 6
	Flour	3
Macaroni	—	21
Millet	—	8
Oatmeal	—	6–11
Oats	—	11 3–16 0
Rice	Polished	14
	Unpolished	44–66
	Milled	16
	Bran	300
	Polishings	284–1,400
Rye	Flour whole	9, 12 2, 17
	" bleached	7 3
Semolina	—	20
Spaghetti	—	21
Tapioca	Boiled	3
Wheat	Whole	28–41, 54–80, 55
	Flour, white	6 9
	National flour—	
	85% extraction (1943)	13 3 24 3, 17
	82½% " (1944)	18
	80% "	16 3
	70% "	8 4–12 0

Food	Description	Nicotinic Acid in micrograms per gram
Cereal Foods—continued		
Wheat—*continued*	Germ	34–70
	Bran	250–460
	Middlings	92–177
	Screenings	192
	U S A , whole	54
	„ white	8
	„ "enriched"	31
Proprietary Cereal Products [264]		
All Bran	Kellogg's	160–185
Bemax	—	60
Cerevim	Lederle (Vitamin concentrate added)	203
Corn Flakes	Kellogg's (Vitamin concentrate added)	16
	Post's (Vitamin concentrate added)	13
Cream of Rice	—	17
Cream of Wheat	Vitamin concentrate added	16 20
Force	—	41
Grape Nuts	Post's (Vitamin concentrate added)	39–49
Oats	Quaker	10
Rice Krispies	Kellogg's (Vitamin concentrate added)	80
Shredded Wheat	Kellogg's	42–45
Soya Wheat	—	34
Vegetables and Vegetable Products		
Asparagus	—	11
Beans	Green	8 5–7 6
	Lima	4
Beets	Root	4–6 4
	Greens	3 0
Broccoli	Leaves	9 , 14 4
Brussels sprouts	—	4
Cabbage	—	1 2–4
	Dehydrated	28 30
Carrot	—	4 , 14 7
Cauliflower	—	4 8 6 6
Celery	—	1 8–2 6
Cucumber	—	1 09–3 2
Egg plant	—	6
Endive	—	7 2
Kale	—	8
Kohlrabi	—	2 7
Lettuce	—	2–5
Mushrooms	—	69 , 31
Onion	—	1 0 5
Parsnips	—	2
Peas	Green	7 21
Peppers	Green	2 0 4
Potatoes	White	11 8
	Sweet	12 9
	Peeled	6 7
	Dehydrated	48
Pumpkin	—	7
Radish	—	1 5 24

Food	Description	Nicotinic Acid in micrograms per gram
Vegetables and Vegetable Products continued		
Soya bean	Flour	7 2 24
	Bread	13 5
Spinach	—	5 7 2
Squash	—	9 6
Tomatoes	Whole	5 8
	Juice	1 0
	Ketchup	20
Turnip	Root	6 9
	Greens	5
Watercress	—	10
Beans and Nuts		
Almond	—	11 7 50
Bean	Kidney dried	17 1 28
	Lima dried	12 7 18 [illegible]
	Broad dried	21
Chestnuts	—	10
Coconut	—	18 2
Cowpeas	—	22
Lentils	Dried	31
Peas	Dried	18 28
Peanuts	Raw	86
Peanut butter	—	162 186
Pecan	—	9
Soya	Bean	22 29
Walnut	—	12
Fruits		
Apples	—	0 9 5
Apricots	—	30
Banana	—	3 6 1
Cherries	—	1 4
Cranberries	—	12 9
Dates	—	8 21 8
Figs	Fresh	10
	Dried	20
Grapes	—	4 8 4
Grape fruit	Juice	1 5 2 1
Guava	—	10
Lemons	—	1 5 1 9
Limes	—	1 0 2 7
Melon	—	8
Orange	—	2 2
Peaches	—	3 9 5
Pears	—	0 9 2 0
Plums	—	1 18 5 6
Pineapples	—	1 36
Prunes	Dried	17
Raisins	—	2 9 6 3
Raspberries	—	3
Strawberries	—	2 3 2 6
Jam	—	2
Dairy Products		
Cheese	Cheddar	2
	Others	1 16
Eggs	Whole	1 0
	Dried	2

Food	Description	Nicotinic Acid in micrograms per gram
Dairy Products—continued		
Milk	Cows, fresh	0 8–1 0
	,, condensed	1 8
	,, skimmed, powdered	6–8 9
	Human	2 6, 1 76 2 45, 1 83 3 3
Cream	—	10
Ice cream	—	1
Meat and Meat Products		
Beef, fresh	Brain	35–49
	Heart	68 84
	Kidney	73 4, 100
	Liver	120–179
	Muscle	46–63 9
	Tongue	71
	Pancreas	58 4
Beef	Extract	375–1 025
	"Corned"	24–95
Chicken	Breast	86, 151
	Muscle, leg	72
	Liver	80–152
Duck	Muscle	30
Frankfurter	—	24
Goose	—	30
Meat extract	—	2 000, 1,000–1 200
Mutton and Lamb	Muscle	45 77
	Brain	32
	Heart	60, 80
	Kidney	60
	Liver	176
Pork	Brain	64
	Heart	40, 73
	Kidney	98
	Liver	140–228
	Muscle	40–61
	Bacon	20–44
	Ham	41, 88
	Loin	88
	Smoked ham	82
Rabbit	Brain	12
	Muscle	65 126
	Liver	143 220
Turkey	—	79
Veal	Heart	106
	Liver	176
	Muscle	65 170
Fish		
Average	Lean	42
Cod	Flesh	23
	Liver	16
	Roe	15 2
Clams	—	10
Crab	—	28
Haddock	—	9
Halibut	—	30–60
Herring	Milt	23 6
	Roe	23 6
	Flesh	29–40

Food	Description	Nicotinic Acid in micrograms per gram
Fish—continued		
Mackerel	—	55 72
Oyster	—	13
Salmon	Fresh	74 84
	Tinned	60
Sardine	—	48 74
Shrimp	—	10 19
Trout	—	35
Turbot	Muscle	23
Miscellaneous		
Aluzyme	—	34
Bemax	—	60
Bouillon cubes	—	up to 270
Chocolate	—	11
Honey	—	0
Coffee	—	100
Malt extract	—	75 134
Marmite	—	600
		527 672
Molasses	—	28 39
Royal jelly	—	59
Tea	—	70
Yeast	Brewer s	300 1 000
	Baker s	400 500
	D C L	250–350
	Torulopsis utilis (food yeast)	400–450

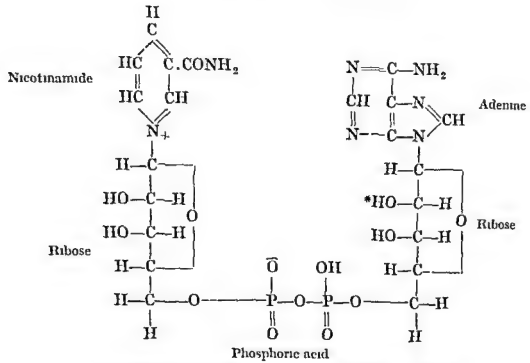
THE PHYSIOLOGY OF NICOTINIC ACID

Nicotinic Acid and Enzyme Systems Nicotinic acid like riboflavine forms part of complex enzyme systems concerned with hydrogen transport in the living cell The enzymes consist of an apoenzyme and a co enzyme The apoenzyme is a specific protein believed to have no enzyme action itself which is linked to the co enzyme the prosthetic group of the enzyme system There are two co enzymes associated with hydrogen transporting enzymes (dehydrogenases) known as codehydrogenases I and II, formerly known as co enzymes I and II

Codehydrogenase I (p 340) or diphosphopyridine nucleotide (DPN) is a complex of one molecule of nicotinic acid amide (nicotinamide) one of adenine two of ribose and two of phosphoric acid [17 18]

ee molecules of phosphoric acid (tri
It is structurally similar to DPN with
acid which is attached to the second
hydroxyl group of the ribose * [319] Both occur in animal and plant cells although DPN appears to be present to a greater extent All living cells can synthesize the codehydrogenases from nicotinic acid Yeast and red blood cells [20] contain relatively large amounts of both Fresh yeast contains about 500 micrograms per gram and human muscle 100 to 400 micrograms per gram Synthesis of DPN from nicotinic acid probably occurs in both the nucleated blood cells [157] and the erythrocytes [48] of the blood In uncomplicated nicotinic acid deficiency there is a decrease in the DPN content of liver and muscle [190]

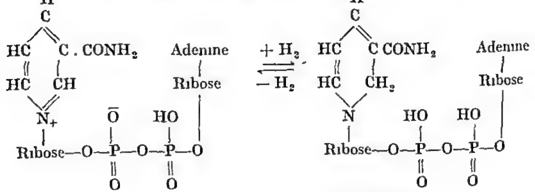
The function of the codehydrogenases is to catalyse the dehydrogenation of various substrates The following table gives some of the dehydrogenation reactions in which they are involved [158]



Codehydrogenase I (Diphosphopyridine nucleotide or DPN)

Substrate and Dehydrogenation Product	Source of Apoenzyme	Codehydrogenase
Lactic acid ⇌ pyruvic acid	Heart muscle	DPN
Alcohol ⇌ acetaldehyde	Yeast	,,
Malic acid ⇌ oxalacetic acid	. . . . .	—
Triosephosphate ⇌ phosphoglyceric acid	Skeletal and cardiac muscle	DPN
2R CHO + H_2O → R COOH + R CH_2OH (aldehyde mutation).	Liver	,,
Formic acid ⇌ CO_2 + H_2O	Seeds and *E coli*	,,
β hydroxybutyric acid ⇌ acetoacetic acid	Heart muscle	,,
Glucose-6-monophosphate → phospho- [illegible]	Yeast, erythrocytes	TPN
[illegible]	Yeast	,,
[illegible]	Liver, yeast	DPN or TPN
Citric acid ⇌ α ketoglutaric acid	Liver, yeast	TPN

During the dehydrogenation the codehydrogenase absorbs two atoms of hydrogen from the substrate to form a dihydro compound. This in turn gives up its two atoms of hydrogen to molecular oxygen, i e. it is oxidized, and codehydrogenase is reformed. The codehydrogenases thus undergo a reversible reduction-oxidation process, the nicotinamide part of the molecule being involved in the change. It is believed that upon reduction the nitrogen of the pyridine ring is reduced from the quinquevalent to the tervalent condition.



DPN Reduced DPN

DPN and TPN are also co enzymes involved in phosphorylation and pyruvic acid oxidation They form part of an oxidation reduction system with the in the metabolism of carbohydrate

to molecular oxygen but requires cytochrome reductase to accept its hydrogen The two flavin enzymes cytochrome reductase and diaphorase (p 293) are catalysts for the transfer of hydrogen from DPN and TPN through the cytochrome system to oxygen DPN and TPN serve as part of the intracellular respiratory mechanism of all cells When coupled with a number of specific proteins they serve for the transport of hydrogen from a number of substances to other respiratory catalysts and make possible the ultimate combustion of metabolites to carbon dioxide and water with the release of energy They function in anaerobic as well as in aerobic metabolism The anaerobic degradation of glucose is catalysed by DPN and adenosine di and tri phosphate (p 194)

According to Wald and Hubbard [43] the conversion of retinene to vitamin A_1 is a reduction in which two atoms of hydrogen are transferred to retinene from reduced DPN This is catalysed by an enzyme in the outer part of the rods of the retina A second enzyme system reduces DPN using hexosediphosphate or one of its derivatives as a hydrogen donor

The content of DPN and TPN in the blood can be increased e g by eighty five per cent by the administration of excessive quantities of nicotinic acid the increase varies with the amount of nicotinic acid administered [20 21] It has been suggested that the determination of DPN in blood might be used as a test for human nicotinic acid deficiency It is however of no in the normal acid therapy Axelrod [23] and his associates determined the DPN content of striated muscle in normal subjects and in pellagrins and they found that it decreases as the pellagra becomes more severe A fall in DPN occurs in heart muscle rendered ischaemic by ligation of the coronary artery [346] Low values of DPN and red hea

to codehydrogenases These derivatives include the salts of nicotinic acid nicotinam nicotinate glucosido monocarb dimethyl carboxylic in place of nicotinic acid [57 60] Not only are these compounds active in doses comparable with that of nicotinic acid (up to 1 000 mg) but they also cause an increase in the concentration of DPN and TPN in the blood It is assumed that these compounds are converted in the body into nicotinamide The nitrogen atom in the pyridine ring must apparently be unsubstituted for the compound to show nicotinic acid activity Thus trigonelline (p 348) in which the nitrogen is methylated is inactive This is understandable as in the codehydrogenases the nitrogen atom of the pyridine ring links up with the ribose moiety of the molecule in trigonelline it is blocked

It is known that sulphonamides such as sulphapyridine and sulphathiazole prevent bacterial growth by interfering with the functioning of chemically related enzyme systems (p 228) West and Coburn [173] noted the chemical similarity between sulphapyridine and nicotinamide—both have a pyridine ring—and reported *in vitro* experiments with *Staphylococcus aureus* on the

basis of which they suggested that sulphapyridine exerts its bacteriostatic effect by interfering with the formation of co-enzymes from nicotinamide. The co-enzyme systems are essential for cell respiration in micro organisms This has been confirmed [174]. Apparently sulphapyridine inhibits the action of nicotinic acid by preventing the formation of the co enzyme systems in which nicotinic acid participates [184] It does not affect the activity of preformed co-enzymes [175] Sulphapyridine not only blocks nicotinic acid in the nutrition of micro organisms, but it inhibits its curative effect in canine black tongue [36] Other sulphonamides such as sulphadiazine, sulphanilamide, sulphapyrazine and sulphaguanidine, are unable to block the nicotinic acid co enzyme systems, presumably because they differ structurally from nicotinic acid in having no pyridine ring [179]

Nicotinic Acid and Porphyrin Metabolism. It has been suggested that nicotinic acid is associated with porphyrin metabolism, since many of the manifestations seen in pellagra, such as abdominal distress, diarrhœa, pigmentation of the skin, and photo sensitivity are often found in patients exhibiting acute toxic porphyrinuria [24] It has been stated that the porphyrin output in pellagra is approximately related to the severity of the skin and mucous membrane lesions and that the excretion returns to normal on a diet rich in yeast and liver and with the regression of the disease [25] It is generally agreed now that the excretion of coproporphyrin in pellagrins lies within normal limits [26, 27, 31] and is of no aid in the diagnosis Rosenblum and Jolliffe [26] have found that the porphyrinuria in pellagra may decrease without the administration of nicotinic acid, or increase with the administration of nicotinic acid, and while the manifestations of pellagra are regressing Liver dysfunction, which is nearly always present in pellagrins, may explain the porphyrinuria of pellagra, either in the form of a disturbance in hæmoglobin breakdown and the production of excessive coproporphyrin, or of the inability of the liver to excrete porphyrin in the bile. Porphyrinuria in old persons has been observed to disappear after the administration of nicotinic acid [176] This may have been due to improvement of impaired liver function

Watson [29] has shown that a colour reaction in the urine of pellagrins, frequently mistaken for that of porphyrin, is in reality due to the pigment urorosein This is present in the urine of many individuals and is not related to nicotinic acid deficiency or pellagra [30, 177]

Nicotinic Acid and Carbohydrate Metabolism. It is now certain that DPN and TPN function in respiratory oxidation systems as carriers of hydrogen and are essential for the metabolism of carbohydrate Fat has a nicotinic acid sparing action, probably because the energy metabolism is shifted from carbohydrate to fat (cf aneurine, p 197) [32] It is possible that more nicotinic acid is required for the metabolism of fructose than of glucose [325] It is known that the energy of nerve tissue is derived solely from the combustion of carbohydrate, and that it is liberated stepwise by means of several enzyme systems, one of which contains nicotinic acid A break in the chain of carbohydrate oxidation may explain some of the mental symptoms of pellagra and nicotinic acid deficiency Pellagrins are stated to show a hypersensitivity to insulin, becoming hypoglycæmic more readily than normal subjects after an injection of insulin [180] This is probably due to impaired liver function and adrenal damage Glucose storage is delayed in pellagrins, who show a "lag" type glucose tolerance test curve, with a blood sugar 15 to 30 mg above resting level even after three hours [273] Large doses of nicotinic acid are said to increase storage of glycogen in the liver of rats [337] The view that nicotinic acid has a hypoglycæmic action in normal subjects and diabetics rests on slender evidence [178 268,] More recent re-investigation shows that neither nicotinic acid nor nicotinamide have any effect on the blood sugar and acetone bodies of normal and diabetic subjects [33, 41, 44] Banerjee and his colleagues [49] state that nicotinic acid has no effect on the blood sugar of normal rabbits, and that

it has no effect on glucose tolerance or on glycosuria Janes and Myers [76] observed ketosis in alloxan treated diabetic rats receiving nicotinic acid Lazarow, Liambies and Tausch [317] protected rats against diabetes produced by alloxan with nicotinamide, Banerjee and his co-workers [49] were unable to show a consistent protection

Whatever the effect of nicotinic acid in physiological quantities is on enzyme systems controlling the metabolism of carbohydrate, the administration of quantities in excess of normal requirements has virtually no effect on the blood sugar in health or in the diabetic.

Nicotinic Acid and Hæmopoiesis. The acidosis and dehydration of dogs suffering from black tongue, which is due to nicotinic acid deficiency, is accompanied by hæmoconcentration If the animals are kept alive with saline injections they suffer from severe anæmia, with red cell counts as low as 0 75 million per cubic millimetre ; the total white count is about 2,500 per cubic millimetre, and the bone marrow hypoplastic Erythropoiesis stops at the erythroblast level Administration of nicotinic acid, or its amide leads to a rapid restoration of the red and white cells to normal [267] Since immature nucleated erythrocytes respire they probably utilize the codehydrogenases Pigs fed a nicotinic acid deficient diet develop a normocytic anæmia [79] The association of anæmia with pellagra has long been known, but since it is a multiple deficiency disease the anæmia cannot be specifically ascribed to nicotinic acid deficiency It is variable in type (p 363) and sometimes responds to iron therapy alone The administration of nicotinic acid to malnourished anæmic subjects raises the pyridine nucleotide content of the blood cells, but has no effect on that of normal subjects [320]

Nicotinic Acid Requirements of Micro-organisms Nicotinic acid or one c[illegible] for a number of organisms, some of which a[illegible] ay of the vitamin (p 334) Some organ- [illegible] group, can synthesize nicotinic acid [illegible]

particular interest, an X-ray induced mutant strain of *Neurospora crassa* (p 344) *B influenzæ* can only utilize nicotinic acid in the form of codehydrogenases Using different organisms it is possible to estimate nicotinic acid, nicotinamide, nicotinuric acid and DPN and TPN in the presence of one another Ni[illegible] and a derivative of [illegible] been employed in [illegible]

Biosynthesis of Nicotinic Acid. It is now established that nicotinic acid is synthesized by organisms in the human gut [266, 269, 302, 303], mainly by *B coli* [265] The action is accelerated *in vitro* by ornithine, but whether this amino acid is essential *in vivo* is not certain According to Ellinger [302] about sixty per cent of the daily [illegible] synthesized by the flora of the hun[illegible] much of this nicotinic acid is abs[illegible] released by the bacteria Najjar [[illegible] little as 1 5 to 2 mg of nicotinic acid an excretion of N′-methylnicotinamide equivalent to that found after the ingestion of food furnishing 20 to 30 mg of nicotinamide According to Denko and his co workers [181] little or no absorption occurs from the large intestine, since on a restricted intake of nicotinic acid the fæces may contain large amounts, and the urine very little Benesch [303] has shown that under aerobic conditions bacterial synthesis of nicotinic acid occurs in gut, but under anaerobic conditions the organisms in the cæcum destroy two thirds of the nicotinic acid He therefore suggests that in the normal cæcum an equilibrium is struck between organisms which produce and those that destroy nicotinic acid Symptoms of nicotinic acid deficiency can be produced in man by the administration of sulphonamides

such as succinylsulphathiazole or sulphaguanidine [38] and by penicillin [301], all of which inhibit the growth of intestinal organisms

Some animals, such as the rat and sheep, do not need endogenous sources of nicotinic acid Intestinal synthesis occurs in the intestine of the rat and some of it is formed independently of bacteria, possibly from tryptophane [42]

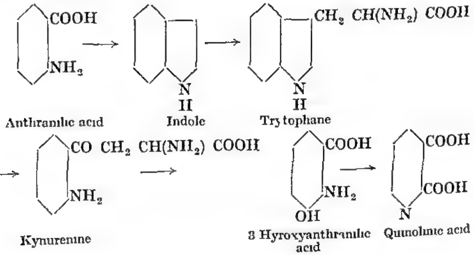
Nicotinic Acid and Tryptophane. It has been known for over 200 years that which maize is eaten as a staple article of fc is a poor source of nicotinic acid, it is not redisposes to pellagra but because this cereal is deficient in tryptophane [77] This explains why foods rich in protein, such as meat and milk, which also contain tryptophane, are effective in the treatment of pellagra. The importance of tryptophane in this connection is that it is the precursor of nicotinic acid. If it is administered in daily doses of the order of 6 gm. it will induce a remission in pellagra in the *absence of other treatment* [82] *and will cause an increased excretion of the* nicotinic acid metabolite, N′-methylnicotinamide. Human adults and infants not suffering from pellagra given 1 to 5 gm of tryptophane daily excrete a much larger amount of N′-methylnicotinamide in the urine than is found under basal conditions [94, 97] At first it seemed that tryptophane exerts its effect through stimulating the bacterial synthesis of nicotinic acid in the intestine [70, 71] The present view is that the conversion of tryptophane to nicotinic acid occurs in the tissues and not as a result of the action of intestinal micro-organisms This is supported by the observation that rats deprived of their intestinal bacteria show no impairment in their ability to convert tryptophane to N′-methylnicotinamide [73] In man the intravenous administration of tryptophane on a constant diet produces a prompt

It also causes a rise in the pyridine nucleotides in the red blood cells ... D Tryptophane is more effective than the L compound [311] Free tryptophane serves as an excellent source of nicotinic acid, but ingested as protein *it is less active* [107] In the rat the excretion of N′-methylnicotinamide is proportional to the casein content of the diet [321], and in the growing animal dietary tryptophane plays a more important part than the nicotinic

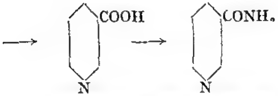
ive as 349],

given in excess it is converted into pyridine nucleotides in the liver and not degraded or excreted [344]

Studies ... strains of the mould *Neurospora crassa* have thrown *some light* n the conversion of tryptophane to nicotinic ac route is from anthranilic acid [66, 342]



Anthranilic acid — Indole — Tryptophane — Kynurenine — 3 Hyroxyanthranilic acid — Quinolinic acid



Nicotinic acid   Nicotinic acid amide

The conversion of 3 hydroxy anthranilic acid to nicotinic acid has been con

of 3 hydroxy

nicotinamide

c [327] can be

used as a substitute for nicotinic acid by the rat [80] Studies with isotopic tracer elements such as C^{14} confirmed the conversion of tryptophane to kynurenine in the intact animal [81] and its conversion to nicotinic acid independently of the intestinal bacteria [325] although the exact stages of the reaction are still conjectural [81] Quinolinic acid may possibly be an intermediate metabolite [168 342] 3 Hydroxy anthranilic acid has been detected in human urine particularly in that of tuberculous subjects [359]

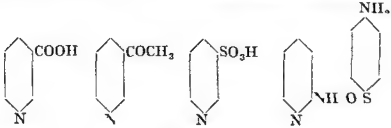
In animals pyridoxine may be essential for the conversion of tryptophane to nicotinic acid and there is evidence that it plays a part in tryptophane metabolism in man [310 311] Animals on a pyridoxine deficient diet excrete considerable quantities of xanthurenic acid after the administration of tryptophane but little N methylnicotinamide or other nicotinic acid derivatives [103] Pyridoxine appears to be necessary for the conversion of tryptophane to tissue pyridine nucleotides [358] It is presumed that pyridoxine functions as pyridoxal phosphate in a manner similar to its role in other transaminase or decarboxylase systems [321]

Riboflavine also appears to be essential for the conversion of tryptophane into nicotinic acid riboflavine and pyridoxine probably function in the conversion of kynurenine to 3 hydroxyanthranilic acid [345]

Nicotinic Acid Antagonists For man

properties of corn have been attributed to a

Its effect can be reversed by giving tryptophane or nicotinic acid A search was made for a pellagra producing compound in corn Woolley [113] obtained a toxamin from corn active in doses of 1 mg 3 Pyridine sulphonic acid and sulphapyridine also act as nicotinic acid antagonists in the dog [115 116]



Nicotinic acid   3 acetyl pyridine   3 pyridine sulphonic acid   Sulphapyridine

In 1946 Kodicek Carpenter and Harris [130] reported that indole 3 acetic acid which is present in a relatively high concentration in maize produces signs of nicotinic acid deficiency in rats But they could not repeat their observations subsequently nor could Rosen and Perlzweig [136] The amino acids *dl* threonine and *dl* phenylalanine can aggravate nicotinic acid deficiency [139]

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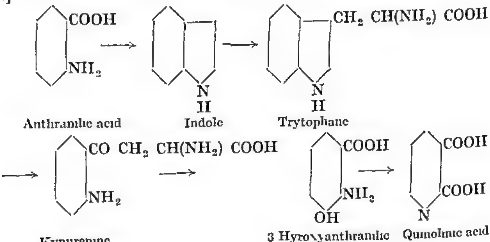
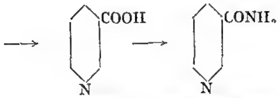
Tryptophane is more effective than nicotinamide and as effective as *nicotinic acid in increasing* the liver pyridine nucleotides of the rat [343, 349], given in excess it is converted into pyridine nucleotides in the liver and not degraded or excreted [344]

Studies with mutant strains of the mould *Neurospora crassa* have thrown some light on the intermediate stages in the conversion of tryptophane to nicotinic acid A possible biosynthetic route is from anthranilic acid [66, 342]



Anthranilic acid — Indole — Trytophane — Kynurenine — 3 Hyroxyanthranilic acid — Quinolinic acid



Nicotinic acid Nicotinic acid amide

The conversion of 3 hydroxyanthranilic acid to nicotinic acid has been con

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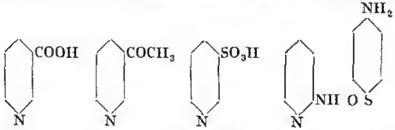
Nicotinic Acid Antagonists For many
properties of corn have been attributed to a
This conception has been supported by the r
In 1945 Woolley [112] demonstrated that the administration to mice of 3 acetylpyridine which resembles nicotinic acid structurally produced a disease with a symptomatology similar to that seen in nicotinic acid deficiency Its effect can be reversed by giving tryptophane or nicotinic acid A search was made for a pellagra producing compound in corn Woolley [113] obtained a toxamin from corn active in doses of 1 mg 3 Pyridine sulphonic acid and sulphapyridine also act as nicotinic acid antagonists in the dog [115 116]



Nicotinic acid 3 acetyl pyridine 3 pyridine sulphonic acid Sulphapyridine

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Pharmacology. The toxicity of nicotinic acid has been studied by Unna [56]. Four to five grams of nicotinic acid per kilo of body weight are necessary to produce acute toxic effects in mice and rats; the amide is twice as toxic in these animals, although in human beings the amide is better tolerated than the acid itself. Nicotinic acid is not toxic to rats, chickens and dogs if taken over a prolonged period (two months) in doses of 2 grams per kilo of body weight. Toxic doses result in ataxia and cyanosis. Symptoms resembling anaphylactic shock have been reported in man after doses of 50 mg intravenously, but the occurrence of such symptoms must be very rare [149]. Temporary leucopenia after administering nicotinic acid has been reported, and this too must be extremely rare [333].

Nicotinic acid possesses a pronounced vasodilator action, which was observed by the earlier workers when they used it in the treatment of pellagra [61–63]. The effects noted both in pellagrins and in normal persons include flushing of the face and neck, a sensation of heat, tingling and itching, which come on within seven to ten minutes and last about thirty minutes. There is light dizziness, thumping in the head, headache, and sometimes nausea, vomiting and transient abdominal pain; the blood pressure is not appreciably affected. An urticarial rash, palpitations, cyanosis of the nails and mental depression have also been described. Reddening and flushing of the skin may occur with either an increase or a decrease in the skin temperature. The symptoms are transitory and harmless, although disturbing. Sebrell and Butler [61] include gastro-intestinal symptoms, substernal oppression, and pruritis among the symptoms. Oral doses of 100 to 300 mg or 20 to 25 mg intravenously will cause an increase of skin temperature. The dose that causes flushing may be as little as 2 mg in susceptible persons, or as much as 300 mg. Bean and Spies [64] have shown that this response is also given by the sodium, ammonium, and monoethanolamine salts of nicotinic acid and by its ethyl ester, and by pyrazine monocarboxylic acid, all of which are effective in the treatment of pellagra. β-Pyridylcarbinol, the carbinol of nicotinic acid, and the tetrahydrofurfuryl ester are also vasodilators [149], but nicotinamide is not [65]. This vasodilator action of nicotinic acid is checked by glycine in doses of 30 to 60 gm and by adrenaline [182].

Loman and his colleagues [137] have shown that nicotinic acid is a peripheral vasodilator, and they infer that its vasodilator action is arteriolar. There is no significant change in body temperature or metabolic rate so that the vasodilatation is presumably not compensatory to increased heat production, but probably to a local effect on the arterioles in the skin [182]. The flushing, itching and heat of the skin, increased motility of the stomach and the secretion of gastric hydrochloric acid that occur after administering nicotinic acid are similar to those produced by histamine. Nicotinic acid however has an anti-histamine action on the bronchi and gut [159].

According to Moore [126] nicotinic acid is a vasodilator of cerebral and spinal vessels, although this is disputed by Scheinberg [170], who observed no change in the blood flow in the vessels. In the rabbit hyperemia of the kidney follows the injection of 10 to 20 mg of nicotinic acid per kilo of body weight [285]. Aring and his co-workers [141] have shown that nicotinic acid and quinine nicotinate administered intravenously increase the rate of intracranial blood flow in human beings for twenty to sixty minutes, without any significant change in blood pressure. Since Moore [126] noted an increase in the width of the pial vessels in the cat after the injection of nicotinic acid, presumably these vessels, at least, are involved in the process, which occurs within several minutes of the injection. The dilator effect of nicotinic acid roughly parallels the reaction of the skin. Nicotinic acid derivatives which do not cause flushing of the skin (e.g. nicotinamide) do not increase the rate of intracranial blood flow.

It was concluded by Popkin [67] that this vasodilator effect of nicotinic acid was too inconstant and evanescent to be of therapeutic value, but

od flow to the hand and 00 to 300 mg of nicotinic effect was due to local changes at the periphery, rather than to an increase in cardiac output, since neither the pulse nor the blood pressure were affected Loman [137] has shown that nicotinic acid can cut short a Raynaud attack artificially produced by the injection of adrenaline into the brachial artery

The vasodilator effect of nicotinic acid in the lower extremity is inferior to that of the drug priscoline (priscol) [169]

Nicotinic acid even at concentrations of 1 in 10^{-6} has a stimulating effect on the isolated frog and mammalian heart [160] In the case of failure of the myocardium it increases the cardiac excursion, reverses abnormal rhythms and augments coronary flow.

The intravenous injection of nicotinic acid in normal subjects is followed by a rise in indirect reaction serum bilirubin, which reaches a maximum in one and a half hours and returns to the initial value in six to eight hours There is also an immediate stimulation and excretion of bile and increased elimination of urobilin, which reaches a maximum in two to three hours and returns to normal in twenty four [40] In pathological conditions of the liver the bilirubin level fails to return to normal values in eight hours and the bilirubin in circulation is of the direct reacting type Both sodium nicotinate and nicotinamide exert a powerful choleretic and cholagogue effect [331]

Nicotinamide, but not nicotinic acid appears to enhance the bacteriostatic effect of penicillin on *Staphylococcus aureus* [172] The effect is not observed with other streptococci or *B coli* On the other hand, it exerts a marked inhibitory effect on the growth of *Mycoderma tuberculosis* [330]

Frankau [183] has shown in a series of carefully controlled experiments, the results of which were submitted to statistical analysis, that the administration of nicotinamide in doses of 50 to 200 mg to active young men results in increased efficiency in carrying out fairly severe tests involving both physical effort and co ordination There was a well marked diminution in the time taken to complete the test and less fatigue in the subjects receiving nicotinamide compared with controls

Shock and Sebrell [163] could not observe any change in the work output of isolated muscle perfused with a dilute solution of nicotinic acid

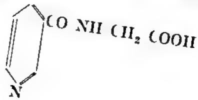
Absorption, Storage and Excretion of Nicotinic Acid. Nicotinic acid is present in foodstuffs mainly as co enzymes from which it is readily released It is not known whether these are absorbed directly as such or hydrolysed to nicotinic acid or nicotinamide first. If nicotinic acid or the amide are given by mouth they are absorbed unchanged Nicotinic acid is converted into the amide after absorption into the blood stream In normal persons the blood nicotinic acid ranges from 260 to 830 micrograms per 100 ml of blood with a On a daily intake of 12 to A rise occurs after severe per cent, is in the blood corpuscles [84] and is present as the co enzymes [186] Ingestion of nicotinic acid causes a rise in the co enzymes in the blood of both pellagrins and normal persons [22] The co enzyme content of the erythrocytes is raised to about three times the normal value on an intake of 200 mg of nicotinic acid daily Ingestion of the amide, however, produces little or no rise in the co enzyme content of blood Large doses of riboflavine do not affect the absorption or storage of nicotinic acid [812]

Nicotinic acid is present in practically all tissues, mainly as co enzymes, the liver contains more than any other organ There is a direct correlation and t the nt in erythrocytes shows only a slight decrease A period of months is required

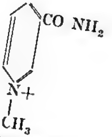
to deplete the body of sufficient of its stores of nicotinic acid to produce pellagra Chronic alcoholics and others with liver damage may have difficulty in storing nicotinic acid [198]

Like other vitamins nicotinic acid is secreted in the milk, which contains about 128 to 336 micrograms per 100 ml [196] It is excreted in the sweat, which contains from 20 to 100 micrograms per 100 ml [187, 188]

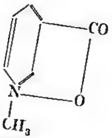
After the ingestion of nicotinic acid or nicotinamide, these compounds and their derivatives appear in the urine The derivatives are the two co enzymes (p 339), nicotinuric acid, N′ methylnicotinamide (nicotinamide methochloride) [229], and N′ methyl 6-pyridone 3 carboxylamide [300] The so called "total nicotinic acid" of some workers includes nicotinic acid nicotinamide, nicotinuric acid and trigonelline According to Holman and De Lange [329] trigonelline plays no part in nicotinic acid metabolism Most of the metabolite described as trigonelline is in fact N′-methylnicotinamide [283] It forms a fluorescent compound with acetone or methylethyl ketone, which serves as a method for its estimation [313] It exerts an anti pellagra action in man [49] It is excreted by glomerular filtration and tubular secretion [304] The substance formerly designated as "F_2" a fluorescent metabolite of nicotinic acid, has been identified as N′-methylnicotinamide It is probably formed from L methionine, or homocystine, which function as methyl donors, and nicotinamide in presence of magnesium ions and a source of phosphate such as adenosinetriphosphate [388] Vitamin B_{12} in the presence of methyl donors increases the excretion of N′-methylnicotinamide [348]



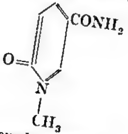
Nicotinuric acid



N methylnicotinamide ("F_2")



Trigonelline



N methyl 6 pyridone 3 carboxylamide

If radio active nicotinic acid is administered to mice only sixty per cent, as measured by radio active methods, can be detected in the urine [274] According to Ellinger and Abdel Kader [214] under normal conditions man eliminates nicotinamide, nicotinic acid and mainly N′ methylnicotinamide They state that extra dietary nicotinic acid or nicotinamide is eliminated almost exclusively as N′ methylnicotinamide, and to a small extent as nicotinamide [214] According to Ellinger and Coulson [283] the mean excretion of N′ methylnicotinamide is 7 5 mg daily, with a range of 2 to 8 mg, this represents approximately 15 per cent of a daily intake of 40 mg of nicotinic acid Fitzpatrick and Tompsett [333] give a range of 2 to 12 5 mg with a mean of 5 8 mg

Methods have recently been elaborated for the estimation of N′ methyl 6 pyridone 3 carboxylamide in urine [292, 295] and it is now possible to account for the major part of a dose of ingested nicotinic acid or nicotinamide

in the urine Holman and De Lange [292] have been able to account for seventy three to eighty per cent of an orally administered dose of 500 mg of nicotinamide in the urine as N methyl 6 pyridone 3 carboxylamide, N methylnicotinamide and the total acid hydrolysable derivatives of nicotinic acid According to Perlzweig Rosen and Pearson [309] 3 6 to 12 mg (mean 8 6 mg) of the 6 pyridone compound is excreted in twenty four hours by subjects on a normal diet They claim that eighty two to eighty nine per cent of an oral dose of nicotinamide can be recovered in the urine as nicotinic acid N methylnicotinamide and the 6 pyridone compound

Many workers have attempted to diagnose nicotinic acid deficiency by estimating various nicotinic acid derivatives in the urine As formerly most workers did not include N methyl 6 pyridone 3 carboxylamide in the excretion products the method has not been of much value in the past Ellinger, Benesch and Hardwick [291] claim that the excretion of methyl nicotinamide after a dose of 100 mg of nicotinamide yields useful information on the nicotinic acid stores of the body The exact extent to which nicotinic acid is synthesized in and absorbed from the gut is not known (p 343) It may be an important source Many factors influence the excretion of nicotinic acid The amount excreted depends upon the intake of protein (i e tryptophane) [94] and on changes in the diet and on drugs [25] Thus it is low on a diet of maize and is diminished by barbiturates poorly absorbed sulphonamides such as phthalylsulphathiazole and succinylsulphathiazole and by drugs exerting a toxic action on the liver According to Ellinger and Coulson [283] the excretion of nicotinic acid is influenced by exercise the presence of methyl donors and the efficiency of the body methylating mechanism It is stated that the excretion in the newborn is greater than the amount present in the milk and that formed by biosynthesis in the intestine [52, 314] This must represent nicotinic acid synthesized by the infant or derived from the mother On the second day after birth the excretion is 3 8 mg and drops to 60 micrograms by the seventh day [142] Smoking is followed by an apparent increase in the excretion of nicotinic acid [53] There is an increase in the excretion of nicotinic acid derivatives in pregnancy [289] and a decrease in typhoid [55] probably associated with increased metabolism and in sprue even when additional nicotinic acid is administered [179] Cayer and Cody [202] observed that the urinary excretion in hospitalized patients with acute and chronic illnesses differed little from those o[illegible]
co work[illegible]
of nicot[illegible]
severe injury, hæmorrhage and infection

HUMAN REQUIREMENT OF NICOTINIC ACID

Nicotinic acid is essential for the nutrition of most animals , it is stated to have a specific growth promoting property [357] It is impossible to give an absolute figure for the nicotinic acid requirement of man This is conditioned by numerous factors including the protein intake, the quality of the protein (particularly its tryptophane content) the presence of nicotinic acid precursors in the diet the possible presence of 'anti pellagra' compounds the relative amounts of other members of the vitamin B complex and the calorific value of the diet Diets poor in protein or containing much corn which is deficient in tryptophane, contain little nicotinic acid The studies of Woolley [112, 113] indicate that the pellagragenic nature of corn may be due to the presence of an anti vitamin (p 345) It is also known that the pyridoxine in the diet plays a part in the conversion of tryptophane to nicotinic acid Bacterial synthesis of nicotinic acid in the intestine is undoubtedly important as an extra dietary source of the vitamin It is possible, as Benesch [303] pointed out, that some intestinal bacteria are con

cerned with nicotinic acid synthesis, while others are breaking it down. Normally a balance is struck between the two processes, but intestinal infections and infestations, diarrhœa, sprue and other gastro-intestinal diseases may upset the balance and increase the need for dietary nicotinic acid.

Dietary studies have shown that diets providing only 3 to 5 mg. of nicotinic acid need not precipitate pellagrous symptoms [281, 282]. It has been estimated that the nicotinic acid intake of Goldberger's volunteers, who developed pellagra in 1913–15 on a diet containing much corn, was 12 mg. daily, whereas the control subjects did not develop the disease on an intake of 7.2 mg. daily (p. 351). More recently Goldsmith and her co-workers [360] have found that 7 mg. of nicotinic acid daily can prevent pellagra even if a diet containing much corn, and hence poor in tryptophane, is consumed. In India the typical rice diet provides from 7 to 9 mg. of nicotinic acid daily, yet pellagra is rare, probably because the protein of rice is of high quality [177]. In contrast, pellagra is rife in Moldavia, where corn is the staple food and the daily nicotinic acid intake is of the order of 15 mg. [286]. This is greater than the 11 mg. daily calculated to be present in the average American diet [204] providing 2,500 calories daily, or the English diet, which contained 9 mg. when the all-white loaf of seventy per cent. extraction was consumed [143]. The present-day diet in Britain probably provides from 11 to 16 mg. of nicotinic acid daily [207]. Very poor diets among some American families are stated to contain only 4 to 6 mg. of nicotinic acid daily [205, 206].

The Food and Nutrition Board of the National Research Council of the U.S.A. in 1948 advised these daily allowance of protein and nicotinic acid:

Subjects	Calories	Protein grams	Niacin mg.
Man			
Sedentary	2,400	70	12
Moderately active	3,000	70	15
Very active	4,500	70	18
Woman			
Sedentary	2,000	60	10
Moderately active	2,400	60	12
Very active	3,000	60	15
Last half pregnancy	2,400	85	15
During lactation	3,000	100	15
Children under 12 years			
Under 1 year	110/2.2 lb.	3.5/2.2 lb.	4
1–3 years	1,200	40	6
4–6 years	1,600	50	8
7–9 years	2,000	60	10
10–12 years	2,500	70	12
Boys and girls over 12 years			
Boys			
13–15 years	3,200	85	15
16–20 years	3,800	100	17
Girls			
13–15 years	2,600	80	13
16–20 years	2,400	75	12

The figures recommended by the National Research Council of America (1948) are given in the accompanying table. These appear to be unnecessarily high if one considers the pellagra-preventive properties of milk, eggs and green vegetables, although in a corn-eating area they may be only just adequate. It is of considerable interest that the Italian pellagrologists of two centuries also recommended a diet containing these foodstuffs for the prevention and cure of pellagra.

Nutritionists in Britain suggest that a daily intake of 14 mg of nicotinic acid is adequate [220], they recommend that the minimal nicotinic acid content of flour should be 1 6 mg per 100 grams, which is approximately the content of eighty per cent extraction flour

It has been estimated that the infant at six months obtains 1 to 2 mg of nicotinic acid daily from its milk and relatively little from other sources This low intake is probably adequate because milk is an excellent source of tryptophane

The Nutrition Committee of the British Medical Association (1950) has suggested the following daily allowances of nicotinic acid

	Daily Allowance of Nicotinic Acid
Children up to 1 year	4 mg
,, 2 to 6 years	6 ,,
,, 7 to 10 years	8 ,,
Adult male doing light work	12 ,,
,, ,, ,, medium work	14 ,,
,, ,, ,, heavy work	17 ,,
Adult females (acc to calorie intake)	8–15 ,,
Pregnancy	10 ,,
Lactation	14 ,,

DISEASES ASSOCIATED WITH DEFICIENCY OF NICOTINIC ACID

PELLAGRA

History. The word pellagra, which is a corruption of the Italian *pelle agra*, meaning rough skin, was first used in 1771 by Frapolli, who insisted that the skin lesions of the disease were precipitated by exposure to sunlight The first comprehensive study of pellagra was made in 1730 by Gaspar Casal, a physician of Oviedo, whose name is commemorated in the lesion of the neck known as Casal's necklace (p 358) The disease was first recognized as a clinical entity in Spain and Italy but it was soon observed in many other European countries In 1864 it was first described in North America, although it was undoubtedly widespread before that time In both Italy and America pellagra became a national menace In 1881 there were 100 000 cases in Italy with a population of 16,500,000, in 1830 five per cent of the population were said to be afflicted The modern history of pellagra starts in 1914 when Funk postulated that pellagra was a food deficiency disease This was confirmed by the classical investigations of Goldberger and his associates in the years 1913–15 Attempts to transmit pellagra by inoculation of healthy persons with the blood, secretions and preparations made from pellagrous lesions failed, thus showing that the disease was not infectious After the discovery in 1937 that nicotinic acid cured black tongue in dogs—a disease showing some resemblance to human pellagra—it was used in the treatment of the latt ... ame apparent, however, that nicotinic acid, ... oms of the disease, was not curative Liver ... complex were found to be more effective

Distribution of Pellagra Pellagra, which is endemic in maize eating

it is now disappearing, less than 100 cases are reported annually, in comparison with numbers running into six figures recorded in the last century

By far the largest number of cases occur in America chiefly in the Southern States among the negroes It has been estimated that in 1938 nearly half a million persons in the United States suffered from pellagra and that 3,500 died from it every year [208] This was an improvement on conditions a

decade earlier, when 7,000 died annually in the Southern States with a death rate of 22 4 per 100 000 of population The latter figure was reduced to 5 1 in 1940 A considerable improvement occurred after 1929 owing to the free distribution of yeast to sufferers and the growing of crops on small holdings by the rural population In the Northern States pellagra occurring among the white population is of alcoholic origin According to Spies [209] one to two per cent of the admissions to the medical wards of Cincinnati General Hospital, Ohio, U S A , suffer from pellagra

Pellagra is also met with in Spain, Portugal, the Balkans Greece and Turkey, South America, India, China, Japan and the Straits Settlements During and after the Civil War of 1937–39 in Spain there was an alarming increase in all deficiency diseases, particularly pellagra 30,000 cases of which were observed in Madrid [145] In Chile there are 3,000 cases annually with a mortality of twenty six per cent [210] Pellagra is rare in Africa, except in Egypt and an area on the east coast The distribution and incidence of pellagra in warm climates is admirably reviewed by Stannus [75] in a series of papers in the *Tropical Diseases Bulletin*

Pellagra is rare in Great Britain, although it is certain that a proportion of the mild cases go unrecognized Before the nutritional nature of the disease was known it was not uncommon to observe cases in mental hospitals and institutions It is still met with in psychiatric practice [211] and sporadic cases are occasionally reported [72] The disease has been reported as a complication of morphine addiction [217] In 1942 Deeny [138] reported on sixteen cases of pellagra in Northern Ireland, where he says mild forms are relatively common and often pass unrecognized, the patient being diagnosed as suffering from neurasthenia, dyspepsia or eczema A patient of one of the authors suffering from mild pellagra was treated for six months for eczema without the true nature of the condition being diagnosed [212]

Ætiology Pellagra is a multiple deficiency disease In 1937, when nicotinic acid was found to be of value in the treatment of the condition, it was at first concluded to be due to nicotinic acid deficiency But nicotinic acid does not cure pellagra Indeed some symptoms of pellagra do not respond to nicotinic acid at all Moreover, there is no correlation between the incidence of pellagra and the nicotinic acid content of the diet There were for example, some inhabitants in Madrid during the Spanish Civil War with a low intake of nicotinic acid, yet they did not get pellagra [203] In any case there is never an uncomplicated nicotinic acid deficiency, if the diet is lacking in nicotinic acid, it is lacking in other factors Pellagra, as it occurs endemically, is a disease with disturbed metabolic relationships involving nicotinic acid, pyridoxine, tryptophane, possibly adenine, amino acids, proteins, aneurine, riboflavine and folic acid It is interesting to note that for many years the belief was widely held that since pellagra was endemic in many maize eating areas, it was caused by a toxin in the grain This was definitely disproved in 1910, when Stannus [75] described an out break of pellagra among African natives on a diet of rice and beans , many cases of pellagra were seen in Japan among white prisoners of war whose diet was low in calories protein and the vitamin B complex

The pellagra syndrome has not been produced experimentally in man by diets deficient in nicotinic acid only Briggs and his colleagues [281] noted a sunburn like erythema in subjects kept for forty weeks on diets containing only 4 mg of nicotinic acid daily there were no other symptoms of pellagra

There are many external factors that play a part in precipitating an attack of pellagra They include the following

Infestations and Infections These include malaria schistosomiasis amœbic and bacillary dysentery, ankylostomiasis and intestinal tuberculosis They operate by interfering with the absorption of food (diarrhœa) increasing the general metabolism owing to pyrexia or by the parasite absorbing foodstuffs from the host

Increased Metabolism It is well known that pellagra occurs in women during pr ng forced labour I riod

Lesion or con ditioned pellagra has been recorded following some lesion in or in operation on the gastro intestinal tract such as carcinoma of the alimentary tract œsophageal stricture gastric and duodenal ulcer chronic gastritis enteritis operations inal fistulæ nutrients

There is an excellent review by Bean Spies and Blankenhorn [261] on secondary pellagra

Alcoholism Pellagra due to alcoholism is not uncommon in the northern

Fig 97 Secondary Pellagra conditioned by Cardiospasm Anterior and lateral views of X ray after swallowing barium meal showing enormously dilated and tortuous œsophagus with a constriction in the middle third Same case as Fig 91

United States Strong alcohol irritates the gastro intestinal tract and causes gastritis and secondary infection which result in faulty absorption of food (cf p 225) The alcoholic consumes calories but not food

Restricted Food Intake The quality and quantity of food consumed may suffer as a result of eating unbalanced diets Food faddists eccentrics asylum inmates and slimming patients have been known to develop pellagra Pellagrins may become insane (p 360) and the insane may develop pellagra because of nutritional failure Gastric disturbances and gastritis are also common in psychotics

Sunlight and Physical Trauma Exposure to sunlight or any physical trauma may be a precipitating cause of pellagra (Fig 98) From a study of 465 cases Ruffin and Smith [85] noted that not only sunlight but radiation from an electric heater will precipitate not only the dermatitis of pellagra but also oral and gastro intestinal symptoms in convalescent patients This was prevented by liver extract Sunlight acts as an irritant exposure to any other form of irritation such as tight clothing repeated friction irritating sweat or friction between body surfaces (thighs nates scrotum) may cause

skin lesions in pellagrins There is a distinct seasonal incidence which is higher in the late spring and early summer Analysis of diets shows that this seasonal wave cannot be explained solely in terms of dietary deficiency [78]

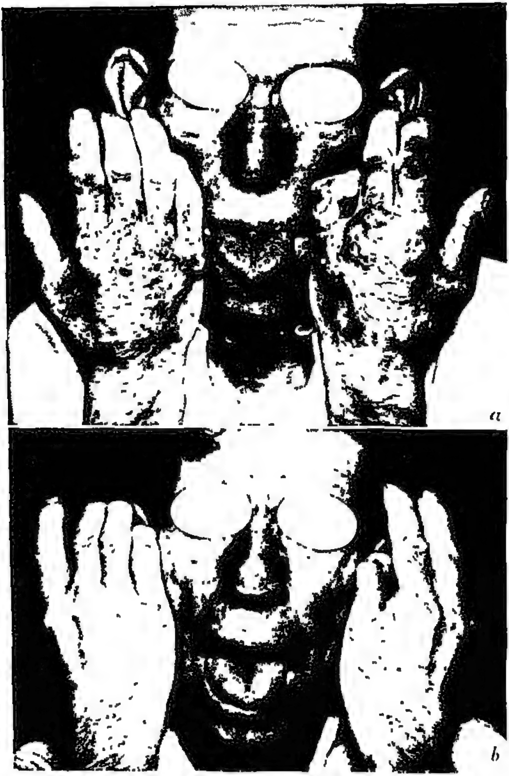
Diets containing much maize or tryptophane deficient protein or unbalanced amino acid mixtures are pellagragenic (p 344) Pellagra does not develop if such diets are supplemented with milk eggs meat and green vegetables Woolley [112 113] has extracted a toxamine from corn which is known to contain a nicotinic acid inhibiting factor [77 230] If the corn is treated with lime or alkali the pellagragenic property is lost [351 352] This is of interest as pellagra is rare in Mexico where much corn is eaten in the form of *tortilla* which is made with lime water A deficiency of adenine may possibly be a factor in the aetiology of pellagra [146]

FIG 98 Pellagra The patient is a labourer wearing trousers but no shirt whilst working in the daytime A pellagrous dermatitis is present on the part of the arms and body exposed to light It is absent over the area protected by the patient's braces Exposure to light may be a precipitating cause of the skin lesions of pellagra

Fatty infiltration of the liver and liver damage is a common finding among pellagrins These liver changes have been extensively studied by Gillman and Gillman [270 276]

Clinical Signs and Symptoms of Pellagra It is apparent from the study of a number of pellagrins that there is a long prodromal period of ill health with insidiously advancing symptoms which at first appear trivial but gradually increase in intensity It takes four to eight months for the full blown manifestation of the disease to appear [322] Loss of weight strength and appetite insomnia vertigo headache dyspepsia anorexia sore tongue and mouth and constipation or diarrhoea are common prodromal symptoms and appear without obvious cause In the preclinical stage constipation is common Irritability loss of memory and depression may be common complaints Other early symptoms include abdominal pain nervousness palpitation flight of ideas inability to concentrate and mental confusion dizziness on rising pains in the limbs and vague alimentary symptoms It is clear that the early syndrome presents no uniform clinical picture and in the early stages a diagnosis of neurasthenia may easily be made The diagnosis of preclinical pellagra can be made if these signs and symptoms are

SECONDARY PELLAGRA



Fig

associated with *grossly inadequate* nutrition, with persons suffering from gastro-intestinal disease or who have been submitted to surgery of the gastro-intestinal tract, or with persons whose vitamin requirements have been increased by pregnancy, lactation, infection, diseases of the thyroid, or increased physical exercise (as in prison camps). At this stage the disease is readily arrested by the *provision of nicotinic acid and other vitamins in high doses*, supplemented by liver, yeast, and an adequate diet.

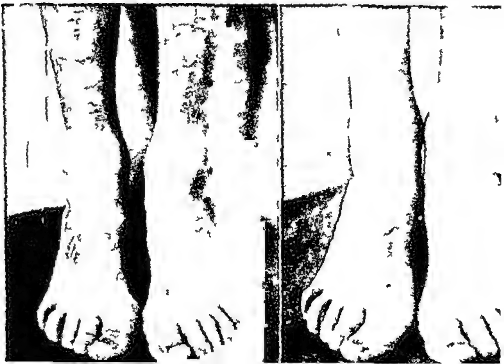
The facies of pellagra often exists before typical manifestations appear. According to American workers, the pupils are usually dilated (Fig 106), the

Fig. 100. Pellagrous Dermatitis. The heavily pigmented and cracked condition of the skin is characteristic. A case treated at the London Hospital.

sclera is bluish and leaden coloured, the eyes and eyelids move slowly, and there is a characteristic dull lifeless stare (Fig. 106) There is an anxious or querulous expression around the eyes, which is so marked that it may some times be of diagnostic aid. The typical pellagrin is profoundly miserable. The ambulant pellagrin has frequently a muddy complexion and a *slightly* pigmented or macular eruption over the face, particularly the alæ of the nose and exposed surfaces of the neck, long before a typical dermatitis appears

General Symptoms These are summarized in the full-blown case in the mnemonic " Diarrhœa, Dermatitis and Dementia." The general symptoms include insomnia, loss of weig
almost any part of the body,
head may feel dull, there may be difficulty in concentrating,

Figs. 101 and 102. Pellagra. A case admitted to the London Hospital under Dr



varying from a sensation of fullness or pressure, localized or general, to boring or stabbing pains are complained of. Any of the prodromal manifestations previously described may be met with So-called "neurasthenic" symptoms occur in the prodromal stage. More than half of all severe cases have a macrocytic hyperchromic anæmia, and a number show achlorhydria Pellagra may present no clear-cut clinical picture The following manifestations are often seen

Gastro-intestinal Symptoms These precede the other symptoms and lesions and are usually the presenting ones Loss of appetite, nausea, indigestion, vomiting, abdominal pain and constipation or diarrhœa are early complaints. Glossitis (Figs 99, 111–113) and stomatitis are among the first symptoms The tongue is indented and fiery red, first at the tip and edges and later the entire tongue is smooth, glazed and denuded of superficial epithelium and papillæ (Figs 112, 113) and is painful and inflamed as in sprue (p 151) Often there are areas of superficial ulceration over the mucous membranes of the tongue and mouth, and smears from these show large numbers of Vincent's organisms. Dysphagia, a scalding sensation in the mouth, increased by highly seasoned foods or hot drinks, may be so painful that the patient refuses food, vomiting may be serious There may be a salty, bitter or bad taste in the mouth and the pain on swallowing may cause increased salivation Œsophagitis is common and food may burn all the way down Œsophagoscopy under local anæsthesia shows a hyperæmic and œdematous mucosa with multiple small punctate ulcerations, and a barium swallow shows up many small constricted areas along the course of the œsophagus [272]

About fifty per cent of pellagrins have achlorhydria even after histamine stimulation In some cases [illegible] in the stomach after treatment with nicotinic ac[illegible]tion of the stomach reveals an atrophic mucos[illegible]ia, hypomotility and retarded evacuation [219]

In the early stages of pellagra the patient may be constipated, it is only later that diarrhœa becomes a prominent feature, although it is by no means constant even in severe pellagra Chronic diarrhœa when it does occur is distressing the patient passing anything from three to thirty stools a day These are liquid, profuse, foul and gaseous, sometimes they resemble those of sprue or those of dysentery, if they contain blood and mucus Frequent defæcation produces a burning feeling in the rectum and proctoscopy shows general inflammation of the mucous membranes The restricted food intake and the diarrhœa lead to emaciation Many pellagrins have an irregular temperature with an evening rise up to 101° F Albuminuria is said to be present in twenty per cent. of the cases

Skin Lesions Pellagrous dermatitis has a characteristic appearance and is distributed in those parts of the body subject to exposure and mild trauma due to tight clothing The lesions are precipitated by exposure to sunlight, fires and radiant heat They are distributed on the face, neck (Casal's neck lace) dorsal surfaces of the hands and lower forearms (Figs 99–103, 105–108) elbows, and the dorsum of the feet and lower legs in bare footed persons There may also be patches over the sternum scrotum, labia and anus and other regions subjected to mechanical irritation or the action of the body secretions These are typical sites, but the dermal lesions may occur on any part of the body They are usually bilaterally symmetrical and are sharply demarcated from the adjacent healthy skin (Fig 105) although Bean, Spies and Vilter [271] have described a number of cases of unilateral or asymmetrical pellagrous dermatitis At first the skin lesions are erythematous and somewhat resemble sunburn but later they change to a reddish brown colour, a fine branny or sometimes coarse desquamation occurs about a fortnight later and the underlying skin is thickened Permanent pigmentation may develop in pellagrins who have been subject to repeated occurrences of dermatitis

The skin becomes scaly and over the legs and hands may *sometimes* present a typical appearance which has been likened to cracked enamel or crazy paving (Figs 83 84) Sometimes the skin is uniformly smooth and shiny (Fig 103) In severe cases the skin over a large area of the body may resemble that of a well roasted turkey

Hyperkeratosis (Figs 109 110) with callus formation is characteristic in chronic pellagrous dermatitis and commonly appears over skeletal pressure points (knee elbow instep front and back of the ankle) and may precede the exfoliating dermatitis and other manifestations of pellagra particularly in those not exposed to sunlight Indeed this may be noted by the potential pellagrin long before the prodromal appearances of the disease This hyper keratosis varies in appearance Over the knees and instep the lesions may be wrinkled or there may be fissures or the hyperkeratosis may be nodular The hyperkeratotic skin commonly shows pigmentation ranging from a light yellow through brown to black

These hyperkeratoses are an exaggerated response to irritation They may also occur on the soles of the feet although they do not necessarily occur over pressure points as they occur on bedridden patients A fairly common type of hyperkeratosis is a diffuse thickening of the skin over the fingers especially over the knuckles it may be smooth and white or fissured rough and pigmented

Another skin manifestation is an ichthyosis like change which may be
apping It is worse in winter than in summer
by exposure to heat The usual site of these
antero lateral aspect of the calves and less
frequently the forearms In a few cases the large thick plaques simulate alligator skin A fine bran like desquamation may also be seen

The skin lesions sometimes become crusted and secondarily infected particularly in natives whose local remedies e g dung usually make the condition worse

The genital and anal regions are often affected and the lesions appear at the same time as those of the tongue and mouth An irritating secretion is poured out by the vagina which may macerate the perianal region The *lesions are red macerated and often infected* particularly with Vincent s organisms These lesions occur in some fifty per cent of females with severe pellagra [222]

In pellagra there is an over activity of
with the formation of inspissated sebum
glands This lesion which according to
cent of pellagrins may

Lesions of Mouth and 113) which
is common is character gins and tip
of the tongue with inde thesia of the
tongue frequently precedes objective signs At times large red fungiform papillae appear against a background devoid of filiform papillae As the *disease progresses desquamation of the superficial epithelium leaves a scarlet* smooth dry and beefy looking tongue (Fig 99) The desquamation may be irregular giving the appearance of the geographical tongue During desquamation secondary infection with Vincent s organisms and monilia
is ultimately shed
hous ulcers develop
he buccal mucosa
gums lips and pharynx producing reddening and superficial ulcerations In advanced pellagra biopsy of the tongue shows extensive fibrosis of the submucosa and the adjacent muscular tissue

The lips are often red and scaly (cheilosis) and fissures appear at the corners of the mouth (angular stomatitis) These lesions are due to riboflavine deficiency and do not respond to nicotinic acid (see p 306)

There is a low incidence of dental decay and caries in chronic pellagrins [340] Spies and his co workers [341] have shown that there is a high content of nicotinic acid in the saliva of patients with severe dental caries and a low content in the saliva of those with sound teeth

Mental Symptoms In pellagrins mental symptoms develop in one third to a quarter of the cases if untreated It has been estimated that in Italy when pellagra was rife four to ten per cent of pellagrins became permanently insane Symptoms are exceedingly varied A feeling of tenseness irritability mental depression and emotional instability are fairly common Patients weep without cause and insomnia is frequent Melancholia lethargy and stupor are common but confused states with hallucinations are also seen as well as excitement mania and delirium The mental symptoms which are often the first to appear are particularly amenable to nicotinic acid therapy

FIG 105 Pellagra A fatal case photographed at Napsburg Mental Hospital Hertford Fre The dermatitis on the dorsal aspect of the hands is bilateral and sharply demarcated from the adjacent skin There are pellagrous lesions on the forehead cheeks chin lips and in angles of the mouth The skin on the dorsum of the forearm is thickened and pigmented The patient was insane and died in a mental hospital

The mental symptoms of pellagra have been specially studied by Frostig and Spies [93] who describe the symptoms of the initial nervous syndrome They are hyperesthesia to all forms of sensation increased psycho motor drive increased emotional drive with a definite trend toward depression and apprehension weariness and increased fatigue headaches and sleeplessness loss of memory and confusion In general the patients appear to have anxiety states with depressive features There are also types in which excitement mania hallucination and delirium may occur A toxic confusional psychosis is very common and a clinical picture resembling Korsakow s syndrome has been described The earlier pellagrologists recorded acute confusional insanity stupor hallucinations acute delirium catatonia manic depressive states and dementia

Psychosensory disturbances occur in all the special senses Patients
ot be tolerated music upsets them
as to cause nausea and vomiting
n edge irritable and tense Many
prominent complaints are dizziness
difficulty in maintaining balance flickering stars and dark spots in front of the eyes

The psychomotor drive is increased—the patient is fidgety moves about a great deal and is quarrelsome He complains that a sudden noise or flash of light makes him jump and twitch Emotional reactions are increased The patient is more excitable and sensitive than usual he is often depressed sad and gloomy an
express various
them The em
danger is constantly expected

In spite of the increased motor drive and restlessness the patients complain of weakness and fatigue They tire readily at their work There is a conflict between restlessness and fatigue with the former often prevailing Sleeplessness is also a common symptom the patient falling asleep between 12 p m and 2 a m and waking again at 5 a m Sick headaches are common resembling those of migraine and occurring suddenly The pain is localized in the forehead and temples and is accompanied by scintillating scotomata As in migraine nausea and vomiting are frequent Developing pellagra often causes a breakdown in personality Individuals previously strong courageous and enduring become shaky weary and apprehensive before clinical pellagra can be diagnosed Severe pellagrous psychoses occur in ten per cent of untreated pellagrins The patient may have periods of depression and apprehension followed by confusion hallucinations delirium disorientation and confabulation A paranoid condition is often observed Tremor jerky movements and rigidity of the body may accompany these symptoms In cases with severe depression the patient may have a mask like expression and sit in one position staring into space for hours without moving

Fig 106 P
toms
and the
the fa
stupor a co

The mental symptoms may precede the other symptoms of pellagra so that a potential pellagrin may easily be diagnosed as neurasthenic or a paranoid This is important because the mental condition clears rapidly in a few days with nicotinic acid therapy whereas a case of true neurasthenia or paranoia remains unaffected Early mental changes are due to biochemical lesions in the brain

Neurological Lesions These may precede accompany or follow the other lesions of pellagra and were well recognized by the nineteenth century

pellagrologists. Pain and cramps in the calves, numbness, burning or itching of the extremities, dizziness, vertigo, tinnitus, formication and other paresthesiae, headache, ataxia, tremors, spastic paralysis, Rombergism, sensory loss, diplopia, nystagmus and absent knee jerks have been reported. Peraita [89] observed the following neurological lesions in pellagrins during the Spanish Civil War: vertigo, diminished visual acuity, acroparesthesiae, causalgia and retrobulbar neuritis. The latter manifested itself in the form of central scotomata and missing letters and interrupted words on attempting to read. Hypohidrosis was also observed [284]. Burning hands and feet are

FIG. 107. Pellagra in a Child. Note the butterfly-like distribution of the dermatitis on the face. Pigmentation is present on the exposed parts and is absent from those covered by clothing.

almost pathognomonic [221]. This symptom was observed by earlier workers and was re-described by medical officers among prisoners of war in Japanese prison camps. The condition is characterized by severe burning and aching of the feet associated with hyperesthesia, raised skin temperature and vasomotor changes in the feet. The pains are more severe at night, interfering with sleep, the patient seeking relief by pacing the room or putting the feet in cold water. The condition became known as "painful feet," "burning feet," "hot feet," and "happy feet," the last named suggesting the dancing movements of the sufferer. Stannus [221] attributed the "burning feet" syndrome to ariboflavinosis, although Gopalan [226] claimed to have relieved the syndrome with pantothenic acid, other members of the vitamin B complex being ineffective.

De Raadt [275] has described a group of oto-neurological symptoms associated with the early stages of pellagra seen in Dutch prisoners of war in Batavia. These include vestibular hyper-irritability, vertigo, tinnitus, subjective deafness, headache, nystagmus, weakness of convergence and lateral gaze. The underlying pathology is a degenerative brain stem encephalopathy. Visual defects were noted by the older pellagrologists, who recorded asthenopia, photophobia, reduction in the visual fields, central scotomata and optic atrophy. These symptoms have been more recently described by Fitzgerald Moore [223], Landor and Pallister [224] and Wilkinson and King [225].

Genito-urinary. Burning on urination, albuminuria, casts and indicanuria may be present. Libido is decreased, sterility common, and menstrual disorders are often seen [228]. Porphyrinuria occurs, but is an inconstant finding and of no diagnostic importance (p. 342).

Cardiovascular. The blood pressure is normal or slightly subnormal, and in severe cases the pulse rate is increased. Death in severe cases frequently occurs after vasomotor collapse and syncope.

Mainzer and Krause [87] studied the electrocardiographic records of a number of pellagrins, and in many the electrocardiogram was abnormal. That these abnormalities had a causal relationship to pellagra is demonstrated by the fact that their development ran parallel to the clinical course of the disease, and by the rapid disappearance in some cases after treatment with nicotinic acid and aneurine. Tachycardia was observed mostly when the disease was at its height, and bradycardia during convalescence. The most frequent electrocardiographic changes were a low voltage and notching of the ventricular complex, inversion of the T wave and shortening of the PR-interval. These changes are, however, not characteristic of pellagra.

Rachmilewitz and Braun [218] also noted marked changes in the electrocardiogram, particularly in the T waves, which were flat in T_1 and inverted in T_2, T_3, T_4, and were reversed by administering nicotinic acid.

Other Manifestations. Anæmia. Anæmia is common among pellagrins although its occurrence is inconstant and it bears no direct relationship to the achlorhydria. The type varies; hypo- and hyper-chromic varieties and various colour indices have been recorded. There is an increased output of urea even on a low protein intake. The polyuria and diarrhœa leads to loss of sodium, he body [324].

Pellagra have been described in which skin lesions The outstanding lesions are stomatitis, the corners of the mouth, and although it is claimed that some cases have responded to treatment with in recent work that these symptoms are

formerly known as infantile pellagra, or kwashiorkor, in Africa is probably not pellagra at all, i.e. not pellagra in infancy. The syndrome is characterized by a failure of growth, œdema, steatorrhœa, lowered plasma albumin, macrocytic anæmia, "crazy pavement" dermatosis, depigmentation of hair, bowel symptoms such as diarrhœa, a deficiency bowel pattern on radiological examination [277] and a fatty liver. The condition has been named malignant malnutrition by Trowell [95], who attributes it to general malnutrition and debility accentuated by tropical parasitic and helminth infections. The crazy pavement dermatosis suggests pellagra, but the resemblance is superficial. It has been suggested by Altmann and Murray [90] that the condition is primarily due to a protein deficiency, although the administration of protein hydrolysates aggravates the condition [91]. Gillman and Gillman [270, 276] reported an excellent response to dried stomach. The fatty changes in the liver, which have been described by Gillman and Gillman, may be due to deficiency of lipotropic factors such as choline and methionine. The late and final results

are hepatic cirrhosis, pancreatic fibrosis and a form of nephritis. Davies [144] suggests that malignant malnutrition is primarily a *pancreatic disorder* due to malnutrition and that in consequence the liver becomes infiltrated with fat.

Diagnosis of Pellagra. The diagnosis of full-blown pellagra is made on the dermatitis, stomatitis, glossitis, mental and gastro-intestinal symptoms, the dietary history and response to the therapeutic test with nicotinic acid and foods rich in the vitamin B complex. The diagnosis is not difficult in cases with a *characteristic pellagrous dermatitis*, especially if this is symmetrical and shows a seasonal exacerbation, but in the absence of the latter, predominating gastro-intestinal symptoms, glossitis and stomatitis may simulate sprue (p. 151). The neuropathy, glossitis and anæmia of pellagra without dermatitis may cause confusion with pernicious anæmia in the tropics and with subacute combined degeneration. The anæmia in pellagra is often *of the normocytic hypochromic* type and the hæmoglobin commonly fifty to seventy per cent. Achlorhydria is common. A history of repeated attacks of the disease, particularly in the spring, and of dietary deficiency helps in the diagnosis. The skin lesions may be mistaken for those of erythema multiforme, erythema solare, occupational dermatitis, syphilis, lupus erythematosis and toxic dermatitis. The nervous manifestations have to be differentiated from those of hysteria, ergotism, lathyrism and general paralysis of the insane. In old people with arteriosclerotic changes and accompanying mental symptoms there may be lesions of the hands and face, which may cause confusion in diagnosis. *Other conditions in which some of the signs and symptoms of pellagra* may appear are tuberculous enteritis, chronic pancreatitis, stomatitis of varying ætiology, neurasthenia and Vincent's angina.

FIG. 108. Pellagra in an English Schoolgirl. The dermatitis is present on the forehead, nose, cheeks, chin, neck and hands. There are lesions at the corners of the mouth as in ariboflavinosis (p. 306). Such cases are seldom seen outside mental hospitals.

The *Plummer-Vinson syndrome* has *some symptoms in* common with pellagra—glossitis, dysphagia and anæmia. The possibility should always be borne in mind that pellagra may be associated with other diseases such as syphilis, tuberculosis, *tropical diseases and conditions mentioned* on p. 352, which may act as predisposing causes, and to which pellagra may be secondary.

Laboratory Tests for Diagnosis of Nicotinic Acid Deficiency. So little is known about the fate of nicotinic *acid in the body and so many* factors influence the excretion of the vitamin that tests for nicotinic acid deficiency

THE SKIN IN PELLAGRA

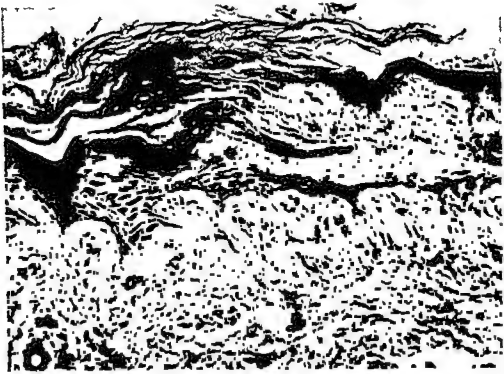


FIG 109 The Skin in Pellagra. Low-power photomicrograph showing hyperkeratosis, œdema and distortion of the rete pegs of the epidermis.

FIG. 110. The Skin in Pellagra Low-power photomicrograph showing hyperkeratosis, atrophy of the epidermis, and œdema of the cutis.

based on excretion tests are of little value although Ellinger Benesch and Hardwick [291] claim that the excretion of methylnicotinamide after a dose of 100 *mg of nicotinamide* reflects fairly accurately the stores of nicotinic acid in the body The method was used as an index of nicotinic acid nutrition in R A F personnel [288] Apparently deficient subjects may excrete only *half as much methylnicotinamide* after a test dose of nicotinic acid as normal subjects but so do subjects from a mixed public ward in a general hospital [286] It is claimed that measurement of the excretion of N methyl nicotinamide for one hour with the patient in the fasting state or for longer periods after a test dose yields helpful information [294 315] Johnson and his co workers [316] claim that an excretion of 0 03 mg or less of this compound in one hour while fasting indicates a subnormal storage in the tissues and that less than 0 5 mg in four hours after the oral administration of 50 mg of nicotinamide indicates deficiency A urinary excretion of less than 95 micrograms per 100 ml per hour fasting is said to be below normal and 45 micrograms or less to denote deficiency [323] Goldsmith and her co workers [360] noted pellagrous lesions in subjects excreting 0 5 to 0 6 mg daily

The excretion of porphyrin in pellagrins is within normal limits and is of no help in diagnosis (p 342)

Blood levels of less than 400 micrograms of nicotinic acid per 100 ml are stated to be below normal and less than 200 micrograms extremely low [323]

Pathology of Pellagra Post mortem the only external appearances that are of diagnostic value are the skin and mouth lesions emaciation occurs late The pathological lesions are frequently obscured by complicating diseases such as bacillary dysentery and tuberculosis

The most striking histological skin changes are hyperkeratosis (Figs 109 110) parakeratosis acanthosis hyperplasia of the sweat glands dilatation of the papillary blood vessels moderate lymphatic infiltration and plugging of the hair follicles with dry sebaceous material [232 233] Slight œdema of the deeper portions of the epidermis occurs and cells of the basal layer undergo multiplication The skin lesions are sharply limited to the zone between the corium and epidermis Generally speaking the microscopic picture is similar to that found in chronic inflammatory diseases of the skin The skin changes are to a considerable extent reversible and may represent a specific response on the part of the skin to a deficiency of nicotinic acid and possibly other members of the vitamin B complex

Degeneration of the nerve fibres of the skin in early pellagra has been described Vesicular formations in the epidermis may occur and if they become infected the epidermis sloughs off leaving an atrophic or horny lamellar corium The pigmentation is due either to the formation of melanin in the malpighian and basal layers of the epidermis or to the formation of granules of an iron pigment in the epidermis In old lesions atrophy and reduction in the cells of the malpighian layer occurs

As a rule little can be seen macroscopically in the gastro intestinal tract Greenfield and Holmes [71] describe the *etat mamelonne* of chronic gastritis enteritis has been described The walls of the colon may be thickened and red and covered with pseudo membranous patches and minute grey cysts formed by distension of the crypts of Lieberkuhn

Vedder [231] states that the gastro intestinal tract is considerably inflamed and frequently ulcerated particularly in the small intestine colon and rectum To what extent these changes are terminal ones following emaciation is difficult to decide Fatty degeneration of the viscera and atrophy of the thyroid and adrenal glands have also been described

The nervous lesions are late T m t common lesions in the spinal cord consist of areas c in various tracts particularly peripheral portions of the fibres are frequently spared In most cases the

afferent tracts are more affected than the efferent, although in occasional cases no lesions in the posterior columns have been observed Chromatolysis of the posterior root ganglia
dorsal, lumbar and lower c
pigmentation of the cells c
appearing to be degenerated from the cervical region downwards The cells in Clark's column are particularly affected In the anterior horns in the lumbar region the cell bodies are swollen and the nucleus is displaced to the periphery as a result of chromatolysis [231] In the brain the frontal lobe is most frequently involved The large pyramidal cells, in scattered foci show chromatolysis with nuclear displacement and accumulations of fat In late cases gliosis occurs There is some wasting of the brain with excess fluid in the ventricles Pathologically the lesions bear some resemblance to those of subacute combined degeneration

Leigh [356] states that there is a retrograde cell degeneration affecting certain groups of nerve cells throughout the nervous system the Betz cells being invariably affected He considers that involvement of the Betz cells is pathognomonic

Prognosis and Treatment Most cases develop in late winter or spring, become more and more severe for two or three months and then slowly improve The patient may recover completely or vague symptoms may remain Recurrences may occur every spring, and with each attack the patient becomes weaker and more emaciated until death occurs, in the average untreated case, in about five years Acute cases have been described in which death may occur in the first attack owing to severe gastric and nervous involvement Recovery readily occurs following effective treatment, but relapse is common

Nicotinic acid, sodium nicotinate, or nicotinamide relieve the acute mental symptoms in a dramatic fashion, and also improve the alimentary tract and skin lesions, but have little effect on the neuropathy, or on the lesions of the lips and face Nicotinamide h
does not produce vasodilatati
reactions (p 346) the dosage
acid Reactions from the latte

Treatment of Mild Cases
recover rapidly on a diet containing adequate quantities of nicotinic acid and other members of the vitamin B complex Plenty of red meat meat extracts, liver, eggs, fresh vegetables, milk and yeast extract or brewers' yeast should be incorporated in the diet Some commercial yeasts may not supply sufficient nicotinic acid to prevent pellagra [278] Small doses of nicotinic acid or nicotinamide, 25 to 50 mg, two or three times daily after meals are helpful Exposure to direct sunlight rough clothing and skin trauma should be avoided

Treatment of Moderately Severe Cases A case of moderately severe pellagra should be confined to bed until the skin lesions have disappeared The same general and dietary treatment as described in the mild cases should be followed Nicotinic acid or, better, its amide is given in doses of 50 to 200 mg daily after food The lesser known members of the vitamin B complex are best administered in the form of boiled yeast (1 to 6 oz daily), or yeast or liver concentrates Crude liver concentrates are given in doses of a tablespoonful three times daily Supplements of vitamins A and D (fish liver oils), C and iron (ferrous sulphate gr 3 to 5 t d s) should be included and a high calorie diet (2 500 to 4,000 calories) provided Riboflavine, 3 to 10 mg daily, and aneurine 5 to 10 mg twice daily, after meals, help to control associated riboflavine and aneurine deficiencies After recovery the dosage of nicotinic acid is reduced to a maintenance dose of 50 mg once or twice daily Pure vitamin preparations are not so effective as an adequate diet

Treatment of Severe Cases. Severely ill patients should be hospitalized and treated as emergencies as they may collapse and die within a day or so. Diarrhœa and dementia are often present so that normal feeding has to be abandoned at first. The patients are usually dehydrated from the diarrhœa and the associated glossitis, dysphagia, anorexia and vomiting prevent the ingestion of food. The administration of vitamin concentrates by mouth is therefore useless in the early treatment of the disease. The patient is given intravenous infusions of five per cent. glucose in normal saline in doses of 500 to 1,000 ml. two or three times daily for the first day or so. This may be continued if the patient cannot take fluids by mouth or has severe diarrhœa. It is not advisable to give yeast at this stage as it cannot be retained and makes the diarrhœa worse. Nicotinic acid or the amide is given in the saline drip in doses of 10 to 20 mg. as a single dose, this amount being repeated at hourly intervals. Too large a quantity at once may cause a reaction. Some workers have found six intravenous injections of 50 mg. a day (total 300 mg. daily) satisfactory. If nicotinic acid is tolerated by mouth and there is no severe diarrhœa, 300 to 500 mg. daily for a week is given orally in divided doses. To the saline glucose drip may be added 100 mg. of aneurine, 50 mg. of riboflavine and 200 mg. of ascorbic acid. Intravenous therapy is continued until the diarrhœa and vomiting have subsided. The patient is then given a liquid diet supplemented by nicotinic acid (300 to 500 mg. daily), yeast, meat extracts, liver extract and aneurine and riboflavine in the doses mentioned. Occasionally patients do not respond readily to nicotinic acid; in such cases favourable results are often obtained by crude liver extracts given parenterally in doses of 5 c.c. daily. Liver extracts and stomach extract are sometimes life saving in severe cases [270]. When improvement has occurred a maintenance dose of nicotinic acid once or twice daily usually suffices. Within one to three days of beginning treatment the fiery tongue and soreness of the anus and vagina subside, the dermal erythema blanches, the acute mental manifestations often vanish overnight, and the papillæ of the tongue regenerate after seven to fourteen days. The diarrhœa however may persist for five to ten days. The mental changes unless of long standing are usually reversible.

Symptomatic Treatment. A mild alkaline mouth wash may be used for the stomatitis, but the teeth should not be brushed as the gums are tender. For the dermatitis dressings of calamine may be used; if there is secondary infection this may require local treatment with penicillin cream or other antibiotics if available, but not with sulphonamides which may produce skin sensitization. Sedatives may be required at first for the uncontrollable mental patients who are however usually amenable after the first few days on nicotinic acid therapy. Tinct. opii 30 minims every four hours and succinylsulphathiazole by mouth help to control the diarrhœa in the early stages. If the hæmoglobin is below fifty per cent. blood transfusions may be given and iron as ferrous sulphate 3 to 5 mg. t.d.s. and yeast administered to control the anæmia. It is important to correct achlorhydria if present as this interferes with the absorption of iron and vitamins.

Relapses are common once the patient passes out of the care of the hospital or physician. This is not surprising. How can the pellagrin whose disease is usually due to poverty afford a diet containing adequate protective foodstuffs? The problem of pellagra is not medical but economic. In America pellagrins are encouraged to keep chickens or a cow or to cultivate kitchen gardens or small holdings. Spies [88] states that a mixture of twenty-five per cent. brewers' dried yeast, sixty-seven per cent. peanut butter and eight per cent. peanut oil in daily doses of 2 ounces is an inexpensive and palatable dietary supplement and tends to prevent pellagra, beriberi and riboflavine deficiency.

Nicotinic Acid Psychoses. In some of the older accounts of typhoid, pellagra, psychotic symptoms were commonly described. It is believed by

Jolliffe and others [99, 100] that nicotinic acid deficiency is responsible for an encephalopathic syndrome which has a mortality of eighty nine to one hundred per cent unless treated A hundred and fifty cases were studied by Jolliffe, who observed that the condition was associated with deficiency diseases, particularly with deficiency of the vitamin B complex The clinical picture is characterized by clouding of consciousness, changing cogwheel rigidity, and uncontrollable sucking and grasping reflexes , there may also be oculomotor disturbances varying from bilateral nystagmus to complete ophthalmoplegia Sydenstricker [101] adds hebetude grading into profound stupor delirium, and agitated depression According to Jolliffe this syndrome may occur independently or in association with pellagra or with polyneuritis or with both Sydenstricker, however, observed it in nineteen cases in the absence of a complete syndrome of pellagra or a history of pellagra

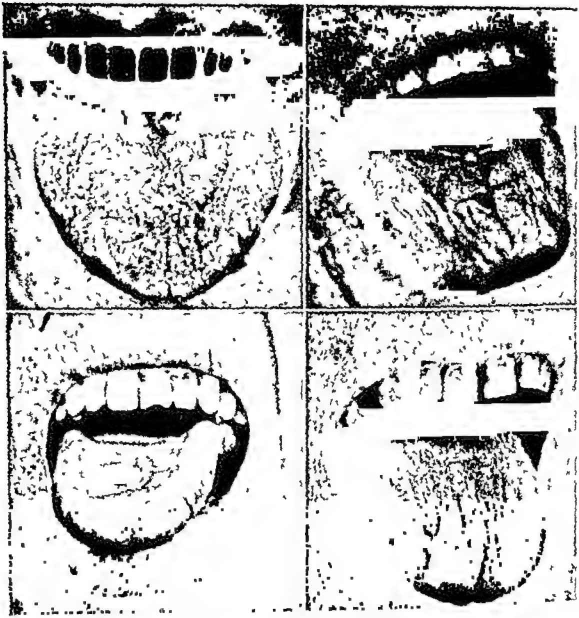
This syndrome treated by hydration and aneurine had a mortality of one hundred per cent in one group and a mortality of sixty two per cent when treated with hydration and the entire B complex In the hands of Jolliffe this was reduced to 31 8 per cent with nicotinic acid, which was injected in 100 mg doses up to a total of 500 mg a day , later, this was increased to 1,000 mg a day in 100 mg doses Sydenstricker used 100 to 300 mg of sodium nicotinate in normal saline containing five per cent glucose, which was given intravenously and 100 mg of sodium nicotinate intramuscularly The cure was described as dramatic Recovery occurred within a few days when the patients were given a high calorie diet supplemented by nicotinic acid and the vitamin B complex, aneurine and riboflavine A control group presenting stupor of demonstrable origin was employed by Sydenstricker they did not respond The workers on this subject are convinced that a therapeutic test with nicotinic acid is justifiable in cases of unexplained hebetude or unconsciousness with a bad dietary history

Jolliffe believes that this encephalopathic syndrome results from a complete and acute nicotinic acid deficiency, while the pellagra syndrome represents a partial and more prolonged deficiency of nicotinic acid Patients showing the encephalopathic syndrome but no signs of pellagra represent a complete nicotinic acid deficiency which develops so rapidly that the changes of pellagra do not have time to occur

Sydenstricker [234] also draws attention to the psychoses formerly classified as ' toxic,' "exhaustion delirium ' and "psychosis, cause undetermined" not infrequently seen in general hospitals and seen sometimes after surgical operations or after delivery In most cases there is no history of frank dietary deficiency, although some patients are alcoholic, some have been dieted for medical or surgical reasons , others have their vitamin requirements increased by fever or infection Intravenous saline and glucose infusions without food by mouth sometimes precipitate an attack The onset of delirium, hallucinations or mania is abrupt, or after a short period of confusion An important diagnostic sign is the fluctuation of the condition, the patient improving or relapsing for no obvious reason The tongue is frequently dry, clean and red—the so called "toxic tongue" Rarely are there any classical signs of nicotinic acid deficiency present Patients, particularly middle aged or elderly, showing mental confusion, delusions hallucinations, stupor manic excitement and confabulation are often admitted to hospital with a provisional diagnosis of uræmia, arteriosclerotic or senile dementia, neurosyphilis, drug intoxication, or a cerebral vascular accident Some are even admitted to mental wards Gottlieb [235] has described several patients admitted to a London hospital under such circumstances

Spillane [147] has observed Arabs picked up in the street unconscious responding dramatically to nicotinic acid The symptomatology included stupor, tremors rigidity and grasping and groping movements Stupor, delirium and acute psychotic symptoms were also seen by Spillane in under

THE TONGUE IN NICOTINIC ACID AND RIBOFLAVINE DEFICIENCY



FIGS. 111 to 114 The Tongue in Nicotinic Acid and Riboflavine Deficiency

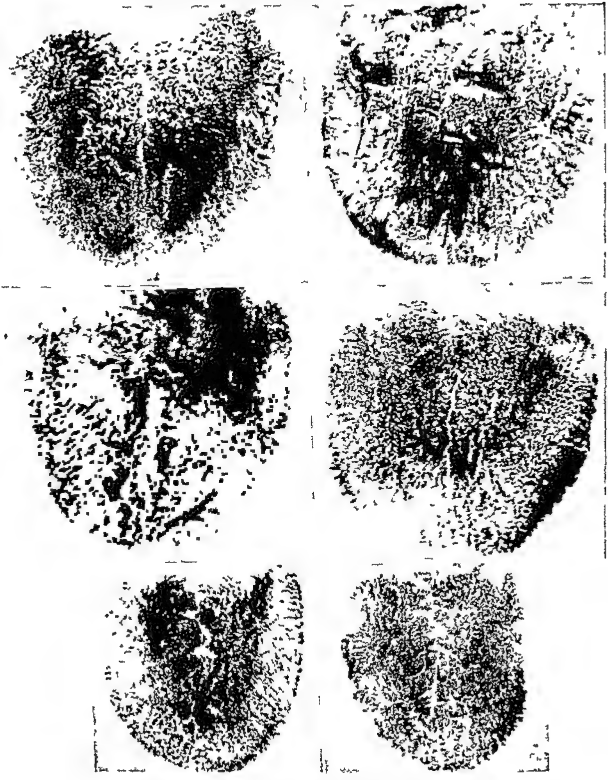
FIG. 111 (*upper left*) Hypertrophy of the papillæ in a patient with nicotinic acid deficiency

FIG. 112 (*upper right*) Tongue, which was fiery red, showing atrophy of the papillæ From a case of nicotinic acid deficiency

FIG. 113 (*lower left*) Bald atrophic tongue due to nicotinic acid deficiency

FIG. 114 (*lower right*) Tongue showing fissuring and hypertrophy of some of the papillæ from a case of riboflavine deficiency. The tongue was magenta coloured

NICOTINIC ACID DEFICIENCY TONGUE PRINTS



Figs 115 to 120 Tongue Prints from Cases of Nicotinic Acid Deficiency

Figs 115 and 116 (*upper left and right*) Tongue print showing progressive atrophy of the papillæ

[illegible]

[illegible] with vitamin therapy

nourished German prisoners of war. Wexberg [98] points out that some cases of senile dementia have nutritional deficiency as a background. These respond to treatment with nicotinic acid, which if given in sufficiently large doses produces a dramatic improvement in the mental condition in twenty-four to forty-eight hours. For immediate treatment Sydenstricker [234] suggests 100 mg. of nicotinic acid or 30 mg. of nicotinic acid amide every hour for ten hours during the first two days, continuing this dosage longer if necessary. This is given by mouth, stomach tube or parenterally if the patient is stuporous or unco-operative. Once improvement sets in the daily dosage is reduced to 100 mg. of nicotinic acid five times a day, or 150 mg. of nicotinamide daily. Later, 25 mg. of nicotinic acid three times daily should be sufficient. Sydenstricker also gives aneurine in doses one-tenth of that of the nicotinic acid, i.e. 50 mg. daily at first. Yeast in quantities of 15 to 30 gm. daily or other sources of the vitamin B complex such as yeast extract or wheat germ preparations are added to the diet.

Mainzer and Krause [121] gave large doses of aneurine in a case of delirium tremens associated with severe gastro-intestinal symptoms without effect, but the administration of nicotinic acid in doses of 500 mg. daily made all pathological manifestations disappear within twelve hours. They believe that the prompt response to nicotinic acid favours the assumption that lack of vitamin is an important factor in the development of delirium tremens. May [122] also observed that nicotinic acid in daily doses of 600 mg. brought [illegible]

psychoses without an obvious nutritional deficiency with nicotinic acid [illegible] to 400 mg. intravenously. Medlicott [299] claimed striking benefit in cases of confusional and schizophrenic psychoses and exhaustion following delirious mania by treatment with nicotinic acid. Lehmann [298] also records a case of confusional state passing into a Korsakoff's psychosis after cerebral injury treated successfully with 25 mg. of nicotinic acid by mouth three times a day and a daily intravenous dose of 50 mg. Gregory [354] treated fifty-four cases of senile psychosis with doses of 300 mg. three times a day orally and 100 mg. daily intravenously with dramatic or considerable improvement in twelve. It is possible that any beneficial effects seen in these cases were due to an increase in cerebral blood flow due to the vasodilator action of nicotinic acid.

Sydenstricker and Cleckley [153] report that thirty-eight patients in stuporous states or in active psychoses without evident cause showed prompt and very often impressive improvement after treatment with nicotinic acid in total dosage varying from 100 to 4,500 mg. orally. Pellagra and other deficiency states were absent. Sydenstricker and Cleckley believe that the symptoms of many cases of toxic psychosis, exhaustion delirium and unexplained clouding of consciousness may be relieved by nicotinic acid. In some cases very large amounts of nicotinic acid (e.g. 4,500 mg.) were necessary to obtain satisfactory results.

Lingual Manifestations of Nicotinic Acid Deficiency. Kruse [96] claims that the examination of the tongue affords a method of detecting nicotinic acid deficiency. He states that atrophy of the tongue, fissures and denudation and reduction of the filiform papillæ occur in nicotinic acid deficiency and that these lesions are reversed by nicotinic acid in doses of 200 mg. daily. Sevringhaus and Kyhos [104] noted that thirty men out of 102 in a prison camp showed the tongue changes described by Kruse, which yielded to treatment with 50 mg. of nicotinic acid daily. While tongue changes may occur as a result of nicotinic acid deficiency, these are not diagnostic of the latter. Upper dentures and iron deficiency anæmia are the commonest cause of denudation of the filiform papillæ; fissures of the tongue can also be congenital and symptomless and are often seen on routine examination of

patients Glossitis may be due to badly fitting dentures and the chewing or smoking of tobacco, it may also occur in sprue and pernicious anæmia In all probability the vitamins are so closely inter related that it is difficult to attribute specific lesions, e g of the tongue, to a deficiency of any one vitamin Glossitis does not necessarily result from pure nicotinic acid deficiency

Bakwin and his co workers [105] concluded that the tongue lesions commonly seen in children are not due to nicotinic acid deficiency

Induced Nicotinic Acid Deficiency Attempts have been made to induce nicotinic acid deficiency in man by sterilizing the gut with sulphonamides or penicillin and thus preventing the biosynthesis of nicotinic acid Hardwick [110] described the development of stomatitis and acute dermatitis after the administration of sulphaguanidine, the lesions disappeared after the administration of nicotinic acid Ellinger and Shattock [148] observed symptoms suggestive of nicotinic acid deficiency in a patient receiving penicillin by mouth, withdrawal of penicillin and administration of nicotinic acid by mouth resulted in cure of the condition There is no proof that these lesions were due to nicotinic acid deficiency, as sulphonamides and penicillin may inhibit the biosynthesis of other vitamins as well as that of nicotinic acid

So far no one has produced a pure nicotinic acid deficiency by omitting nicotinic acid alone from the diet Several workers have described the results of a vitamin B complex deficiency but which members of the complex were responsible for the symptoms is not known

NICOTINIC ACID THERAPY

Nicotinic acid has been used in the treatment of a number of clinical conditions some associated with deficiency disease, while others are not In the latter group it presumably acts pharmacologically and not as a vitamin Nicotinamide can be used instead of nicotinic acid, and in the same dosage, in the treatment of conditions associated with defective nutrition It is in fact preferable to nicotinic acid if the latter is to be injected or given in large doses since nicotinic acid is liable to produce flushing and vasodilatation which although not harmful may prove alarming to the patient (p 346) Nicotinamide is free from these side effects If nicotinic acid itself is given, it is best to keep the single dose within 50 to 100 mg after meals to avoid the vasodilator effect When nicotinic acid is given for its vasodilator action it cannot be replaced by nicotinamide, which is devoid of any such action

Oral Conditions "Trench Mouth" Vincent's organisms are commonly found in the buccal lesions of pellagra and disappear with nicotinic acid therapy King [111, 151, 239] has attempted to demonstrate an association between nicotinic acid deficiency and the syndrome variously described as "trench mouth" Vincent's disease ulcerative gingivo stomatitis and fuso

spiro

pale

marg

and

symptomatology, but differn

Nicotinic acid in doses of 200 mg daily, with a maintenance dose of 100 mg for seven to fourteen days was stated to be effective in the fulminating type, but less satisfactory in the subacute and "flaring" types King states that he found the best treatment to be local application of hydrogen peroxide and

chromic acid and nicotinic acid by mouth. Ascorbic acid therapy was disappointing. He states that most cases clear up given nicotinic acid, riboflavine and ascorbic acid. Good results with nicotinic acid therapy in the treatment of trench mouth are also claimed by Miller, Greenhut and Roth [243], Schwartzman and Grossman [244] and Smith [245].

These views have been criticized by other workers. Ungley and Horton [241] noted that eighty-five per cent. of a group of British naval ratings suffered from trench mouth, but they were unable to relate the incidence of this to nicotinic acid or ascorbic acid deficiency. Nicotinic acid 500 mg. daily

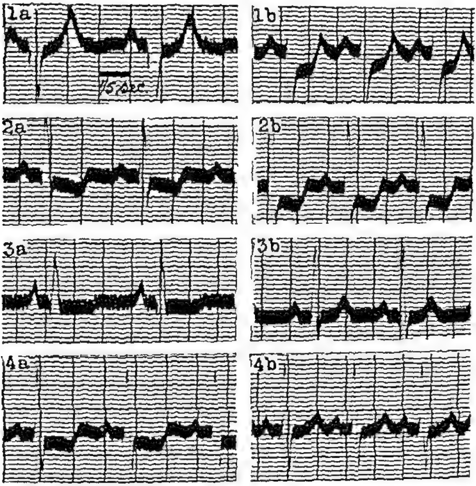


FIG. 121. Electrocardiograms in a Patient with Cardiac Ischemia after Administration of Nicotinic Acid and Glyceryl Trinitrate

(1a) At rest (1b)
(2a) At rest (2b) d which failed

(3a) At rest (3b) which caused flushing

(4a) At rest (4b) 4 minutes after chewing glyceryl trinitrate gr. 1/100

caused no improvement. Cuthbert and Williams [242] and Stammers [239] also found no evidence of nicotinic acid deficiency in trench mouth, nor did they find that nicotinic acid without local treatment had any effect on the course of the disease. Stammers' conclusions were based on a study of over 1,000 cases.

Coulson, Ellinger and Smart [288] examined the nicotinamide methochloride excretion after a test dose of nicotinamide, which is considered to be an index of nicotinic acid nutrition (p. 360), in a number of R.A.F. personnel with normal gums and found it to be higher than in those with various types of gingivitis. The difference was statistically significant, but was not considered to be of ætiological importance since other factors have a marked

gingivitis was present or not. Cocker and Bigger [108] conclude that gingivitis can be adequately treated by local treatment without vitamins.

Cardiovascular Diseases. It has been suggested that the vasodilator action of nicotinic acid might be utilized in the treatment of peripheral vascular disease, especially in
112, 113, 137]. The effective
20 to 25 mg. intravenously.
are of short duration. Lon
nicotinic acid cuts short a Raynaud attack produced artificially by adrenaline injected into the brachial artery. Green and Salber [154] state that a considerable improvement occurred in a case of hemiplegia treated with 150 mg. of nicotinic acid three times daily for fifteen days, and Furtado [246] claims that a dose of 50 to 200 mg. daily gave considerable relief in a case of cerebral thrombosis. As only single cases were reported and both conditions often result in natural recovery, it is difficult to comment on this form of treatment.

Moncrieff [162] has used nicotinic acid with good results in angina pectoris. Neuwahl [247] found that the administration of nicotinic acid by mouth decreased the severity and number of attacks of angina pectoris in a number of cases, but in some the effect was only transient. He, therefore, gave it in the form of an intravenous drip of a 0 05 per cent. solution in isotonic saline. One infusion of 100 to 300 mg. produced beneficial results, which were maximal in twelve to twenty-four hours. In most cases a course of six infusions spread over three weeks was given. Six cases showed complete or almost complete regression of symptoms over a period of three to seven months after treatment. The nicotinic acid was stated to produce a fall in blood pressure and slowing of the heart. According to Stokes [260] the changes in the

effect reported by others of nicotinic acid in the treatment of angina. In ten cases of angina that were all relieved and prevented by glyceryl trinitrate only three received slight benefit from the administration of nicotinic acid in

inferior to theophylline in the

It is claimed that the carbinol of nicotinic acid, β-pyridylcarbinol, is an effective vasodilator given by mouth. It has been used in the treatment of peripheral vascular diseases such as arteritis, acrocyanosis, arteriosclerosis and intermittent claudication [332, 361] The furfuryl ester of nicotinic acid (trafuril) is also an effective vasodilator and has been used locally in the form of a cream for similar conditions.

Skin Disea
nicotinic acid
dermatoses
with nicotinic
and acrodynia
acid alone
with 50 mg. of nicotinamide twice daily for several weeks and reported promising results. It is difficult to understand what effect the nicotinamide had, as it is devoid of any vasodilator action. Nicotinic acid has been used for the treatment of chilblains, in doses of 50 mg three times a day [145], with apparently good results.

Ferreira-Marques [117] states that nicotinic acid and its amide have an antipruritic action. He claims to have effectively treated 150 patients with

EFFECT OF NICOTINIC ACID ON SKIN LESIONS IN A DIABETIC

Fig 122 Diabetic with scaly red indurated and dry skin lesions before treatment

Fig 123 Same patient as in Fig 122 after treatment with nicotinamide 250 to 450 mg daily No local treatment was given and the diabetes was uncontrolled

EFFECT OF NICOTINIC ACID ON SKIN LESIONS IN A DIABETIC

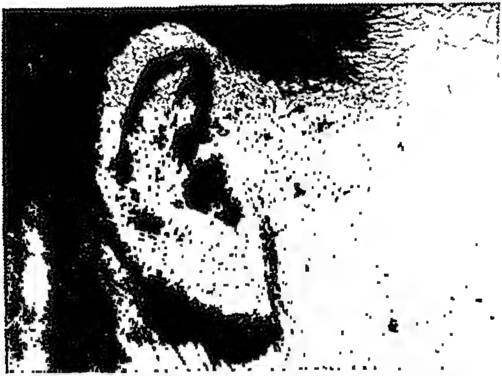


FIG. 124 Diabetic with red, indurated, dry and scaly skin eruption of the ears of three years duration There were deep fissures behind the ears and lesions on the breasts as in Fig 122, and in the pubic, intergluteal, sacral and olecranon regions.

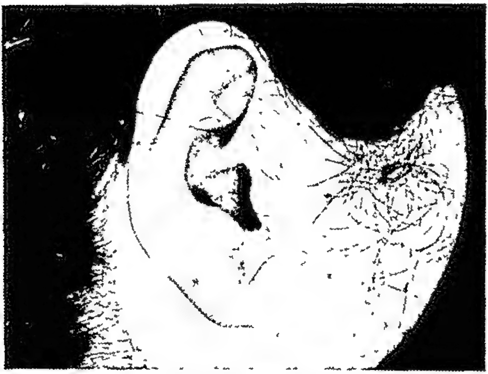


FIG 125. Same patient as in Fig. 124 after treatment with nicotinic acid 200 mg daily. The diet and dosage of insulin remained unchanged.

nicotinamide, sixty-eight of them carefully controlled, suffering from simple pruritus, pruritus vulvæ, pregnancy pruritus, senile pruritus, Hebra's prurigo, Besnier's prurigo, Fox-Fordyce's disease, and pruritus and prurigos caused by irradiation The dose was 200 mg of nicotinamide five times a day The same author has treated lichen planus with penicillin and nicotinic acid [307] and tuberculous skin lesions with nicotinic acid, riboflavine and iron [308]

Nicotinic acid in doses of 50 to 200 mg. four times daily is stated to relieve the pruritus and improve the cutaneous manifestations of dermatitis herpetiformis [213] This is ascribed to the pyridine ring in nicotinic acid since improvement also occurs with sulphapyridine but not with other sulpha drugs not containing the pyridine ring. β Pyridyl carbinol, the carbinol of nicotinic acid, is also effective [350]

Harris and Derian [133] have pointed out that chronic bromide intoxication shows marked parallelism with the symptomatology of pellagra They claim that treatment of bromism with large doses of nicotinic acid causes rapid disappearance of symptoms

Diabetes. Neuwahl [178] claimed to have observed a well marked temporary improvement in the carbohydrate tolerance of diabetics being treated with nicotinic acid for vascular disease He states that further investigation on a group of twelve diabetics showed that the administration of nicotinic acid or nicotinamide diminished the requirements of insulin needed to keep the blood sugar of the diabetics within normal limits The dosage was of the order of 500 mg three to five times daily to begin with, the dose being subsequently reduced as the blood sugar came down The nicotinic acid and nicotinamide were given in enteric coated tablets

Skin disturbances such as pruritus, dermatitis and intertrigo are common in diabetics, and owing to dietary restrictions some degree of avitaminosis may result Rudy and Hofmann [250] state that these skin disturbances are most frequently due to vitamin deficiencies, particularly of nicotinic acid rather than to disturbed carbohydrate metabolism as was formerly thought Pellagrous dermatitis in diabetes is often seen and is sometimes diagnosed as psoriasis Rudy and Hofmann have treated the skin lesions in a number of diabetics by the administration of nicotinic acid or nicotinamide (Figs 122–125) They state that complete cure may take from a few days to a few months, and that more stubborn cases require large doses parenterally as well as orally The dosage given was from 150 to 300 mg daily in divided doses The vitamin B complex was also given in the form of yeast

Asthma Maisel and Somkin [251] first published a preliminary report on the treatment of asthmatic attacks with nicotinic acid Severe attacks were controlled by the slow intravenous injection of 100 mg of nicotinic acid, which was stated to produce improvement lasting from three to fifteen hours Some chronic patients were improved by oral medication, 200 mg three times daily and on retiring The patients noted flushing after administration of the nicotinic acid followed by the expulsion of tenacious mucous plugs The beneficial effect was attributed to a vasodilator effect on the blood vessels or relief of bronchospasm. Neuwahl [252] noted improvement in four cases, but three were made worse Melton [253] gave nicotinic acid in doses of 50 to 100 mg, usually intravenously, to nineteen cases of asthma during acute paroxysms and obtained definite improvement in sixteen cases, two had marked exacerbations The tests were controlled by injections of sterile water Nicotinic acid was also given over a long period in doses of 50 mg two or three times daily and 100 mg at night to thirty cases and the frequency of attacks was stated to have been reduced in sixteen Relapse occurred after discontinuing the nicotinic acid The fact that it makes some cases worse should be borne in mind

Neurology. Selfridge [123, 124] used nicotinic acid nicotinamide and sodium nicotinate in the treatment of some thirty cases of high tone deafness, and in many the results were said to be striking He believes that an under

lying nutritional deficiency explains the nerve changes and all cases observed by him gave a history of faulty diets He states that both aneurine and nicotinic acid gave a response in the hearing curve but that the greatest improvement came from the use of nicotinic acid

Harris and Moore [125] have described the treatment of twenty cases of Menieres syndrome with 250 mg of nicotinic acid and 20 mg of aneurine a day seventeen became entirely free from vertigo They point out however that treatment may have to be continued for several months to obtain complete relief Atkinson [119 161 280] has also used nicotinic acid in the treatment of certain types of Meniere s syndrome He states that patients suffering from this condition may be divided into two groups by an intra dermal histamine test There is a small group sensitive to histamine that can be treated group insensitive to histamine vasospasm In this group Atkinson odilator drugs the most satisfactory of which was nicotinic acid He gives an initial dose of 30 mg intramuscularly and if this is tolerated a dose of 25 to 30 mg intra venously This is repeated daily or every second day for six to eight doses increasing the dose by 5 mg each time to the maximum tolerated which is usually 50 mg but may be as high as 75 mg After a few days oral treatment is started as well usually 50 mg two or three times daily Following intra venous therapy intramuscular therapy is started and given daily for one to three months and then successively decreased to five four three two and one administrations a week At the same time 100 to 150 mg are given daily by mouth After several months treatment oral therapy alone is tried Atkinson believes that nicotinic acid deficiency may result in nerve deafness and tinnitus and ariboflavinosis in middle ear deafness

Atkinson [296] has also treated migraine with nicotinic acid on the assumption that some types are due to a primary vasospasm Treatment begins with the intravenous injection of 25 to 35 mg of nicotinic acid intra muscularly the dose being increased 5 mg daily until a dose of 50 mg is reached Seventeen patients out of twenty one obtained complete relief or improved These results were confirmed by Grenfell [118] but not by Fried man and Brenner [305] Atkinson [297] has treated over 200 cases of tinnitus aurium with nicotinic acid From fifteen to fifty five per cent were relieved and from fifty two to eighty five per cent improved

Goldzieher and Popkin [123] have treated 100 consecutive patients with severe headache due to various causes with intravenous injections of nicotinic acid Seventy five were completely relieved with doses of 100 mg the relief appearing to be correlated with the degree of flushing produced Good results were obtained in the treatment of migrainous headache and in that following spinal puncture Liedholm and Radner [134] observed improvement in patients with headaches following encephalography treated with intravenous sodium nicotinate The well known malarial headache which is exceptionally severe is relieved by nicotinic acid in oral doses of 50 to 100 mg [129]

The surgical treatment of trigeminal neuralgia is not completely devoid of risk and any medical method of treatment is worth investigation Adams and Robinson [120] of Leeds have reported that the paroxysms of the disease can be relieved by nicotinic acid in doses of from 50 mg twice daily to 75 mg four times daily Furtado and Chicorro [254] also used nicotinic acid because they had observed that the pain of trigeminal neuralgia is often accompanied by vasomotor changes in the trigeminal area Their method is to give a daily intravenous injection of 100 to 200 mg In four cases out of eight a few injections gave relief lasting for months and when an injection was given during a paroxysm the relief of pain was immediate

Moore [126] has treated cases of disseminated sclerosis with injections of 60 to 160 mg of nicotinic acid and 33 mg aneurine two or three times

to influence the cerebrospinal fluid pressure and does not dilate the retinal vessels, which are comparable to the pial vessels.

Other Uses. Graham [135] has used nicotinic acid in the treatment of seventy patients undergoing X-ray therapy Only those suffering from severe nausea and vomiting were given nicotinic acid in doses of 30 to 200 mg three times a day. The results obtained were stated to be better than with other forms of treatment. Similar claims are made by Kepp [131], who treated his cases with 500 mg of nicotinamide daily Bean, Spies and Vilter [279] state that patients on diets poor in the vitamin B complex readily develop irradiation sickness, which can be prevented or relieved by administering nicotinic acid or aneurine a few days before exposure Well fed patients had little reaction to the same dose of X-rays that made patients deficient in the vitamin B complex sick. The same authors record a case of classical beriberi and pellagra developing after irradiation therapy They suggest that the basic disorder in irradiation sickness is a disturbance in the respiratory enzyme systems, of which nicotinic acid, aneurine and other members of the vitamin B complex are components The excretion of urinary pigments and DPN and TPN after irradiation therapy is similar to that observed in pellagrins As the diet is often already deficient in patients needing radiotherapy, it would appear rational to supplement it with the vitamin B complex before commencing treatment

Owing to the low excretion of nicotinic acid and its derivatives in surgical patients suffering from hæmorrhage, burns, infection and trauma, it has been suggested that supplements of nicotinic acid of the order of 500 mg a day be given at first reducing this dose to 75 mg daily [127, 128]

Since sulphonamides and antibiotics, such as penicillin, administered orally destroy the bacteria that synthesize nicotinic acid in the gut, increased amounts of nicotinic acid are often administered when these drugs are given for any length of time.

equivalent in anti-tubercular activity to 1 mg of streptomycin ... daily over the same test period It would be impossible to give doses of nicotinamide of this order to human patients Farber and Miller [256] have shown that patients with tuberculosis often show evidence of nicotinic acid deficiency which responds rapidly to diet and specific treatment with nicotinic acid Isonicotinyl hydrazine, a derivative of an analogue of nicotinic acid, is a powerful tuberculostatic drug that has recently been introduced for the treatment of tuberculosis [347] Unfortunately it readily produces drug resistance

Rachmilewitz and Braun [257] made serial electrocardiographic examinations in fifty cases of typhoid fever, and in thirty five there were changes in the T waves, which became flat iso electric or diphasic On the supposition that the cause of

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CHAPTER VI

ASCORBIC ACID

(VITAMIN C)

HISTORY

SCURVY has been a menace to seafarers explorers and armies since classical times although the first recorded accounts of the disease are to be found among the writings of the physicians who accompanied the crusaders in the thirteenth century The decline of scurvy in Europe coincides with the introduction of the potato in the early seventeenth century and the increased consumption of vegetables and fruit Vasco da Gama, who sailed round the Cape of Good Hope in 1498 describes how a hundred of his crew of a hundred and sixty perished from scurvy at sea Sir Richard Hawkins' Observations in his Voyage to the South Sea (1593) contains an account of the prevention and treatment of scurvy with lemon juice Jacques Cartier (1535) during his exploration of Canada found that the native Indians prevented the disease by drinking a decoction of pine needles which we now know contains ascorbic acid

Lind in his famous book A Treatise on the Scurvy (1757) mentions the value of fresh citrus fruits and green vegetables in the treatment of scurvy He gave patients suffering from the disease various forms of treatment including two oranges and a lemon a day Only those receiving the oranges and lemons showed any improvement. During his voyage round the world between 1772 and 1775 Captain Cook kept his men free from scurvy by including in their dietary as much fresh food as possible including fruit and vegetables In 1804 regulations were introduced into the British Navy providing all ratings with daily rations of lemon juice and similar provisions were made by the Board of Trade in 1865

To day frank scurvy is almost a disease of the past It is still met with however in isolated parts of the world and during wartime During the war of 1914 1918 outbreaks occurred among British troops at the siege of Kut and several cases occurred among civilians in Glasgow Newcastle and Manchester owing to a shortage of potatoes

Modern nutritional work on scurvy dates from 1907 when Holst and Frolich of Christiana tried to produce beriberi in guinea pigs by means of restricted diets but scurvy resulted instead Henceforth the guinea pig was used in all experimental work on scurvy After Funk s postulation in 1912 of a scurvy preventing or antiscorbutic vitamin vitamin C attempts were made to isolate it from orange and lemon juice Between 1924 and 1929 Zilva [1] and hi [illegible] concentrate from lemon juice In 1928 [illegible] hich he called hexuronic acid from cabb [illegible] it during the course of studies on cellular oxidation In 1932 Waugh and King [2] isolated it from lemons and identified it with the hexuronic acid of Szent Gyorgyi In the same year Waugh and King [4] showed that the antiscorbutic activity of hexuronic acid was identical with that of the vitamin C obtained from orange juice

The structural formula of vitamin C was established in 1933 as a result of the work of Haworth Hirst and their co workers [6] Karrer [7] and a number of other investigators In the same year Reichstein [8] and his colleagues in Switzerland synthesized the D and later the L form of the

vitamin Almost simultaneously Haworth and Hirst [9] synthesized it in Britain In 1933 the name ascorbic acid was suggested for vitamin C, and this is now the pharmacopœial one It is the L form

CHEMISTRY OF ASCORBIC ACID

Ascorbic acid forms colourless crystals melting at 190°–192° C, with a specific rotation of + 23° in water and + 48° in alcohol It is freely soluble in water, and slightly soluble in acetone and the lower alcohols, but insoluble in benzene, ether, chloroform and fats. It condenses with aldehydes, acetone and other ketones in the presence of mild dehydrating agents to form stable crystalline deri ... lts through an enolic hydrogen atom

If kept dry ... bic acid is stable for a considerable time Vi ... it have a markedly destructive effect; hence it is stored in yellow coloured bottles In solution stability

Fig 126 Crystals of Ascorbic Acid

depends upon many factors. The presence of traces of iron and copper ions (e g 20 micrograms per litre) rapidly catalyses its oxidation Ascorbic acid is stable in the presence of aluminium and stainless steel In aqueous solutions below pH 7 6 it is not oxidized on exposure to air unless traces of copper or other such catalyst are present The rate of oxidation is directly proportional to the square root of the concentration ... tional to the square root of the pH [38] ... destruction is rapid and complete when ... Autoclaving at 120° C for twenty minutes in oxygen at pH 8 results in a loss of forty nine per cent , in ... per cent at pH 8, and four per ce ... that solutions of ascorbic acid can be ... unts of fruit acids such as tartaric and citric acids In foods factors are present inhibiting oxidation [3] Numerous substances such as tissue extracts, glutathione, cystein, thiourea, carbon monoxide, cyanides and halide ions inhibit oxidation Solutions of ascorbic acid and its sodium salt are quite stable if kept in air free vessels or in an inert atmosphere of nitrogen or carbon dioxide The first oxidation product is dehydroascorbic acid, which

can be reduced to ascorbic acid by hydrogen sulphide, cysteine and glutathione

Dehydroascorbic acid L Ascorbic acid

as potent an antiscorbutic as ascorbic acid itself
same way as ascorbic acid, which it can replace

a number of organic compounds
phenol dyes, which are used in
it, two atoms of iodine reacting
of alkali, iodine quantitatively
oxidizes it to oxalic and trihydroxybutyric acids

Estimation of Ascorbic Acid [5] The reactions of reduced ascorbic acid with 2 6 dichlorophenolindophenol, the blue colour of which is discharged and of dehydroascorbic acid with 2 4 dinitrophenylhydrazine are most widely used for the estimation of ascorbic acid in foodstuffs and body fluids Before assay the ascorbic acid must be extracted with one to two per cent oxalic acid, or metaphosphoric acid, five to ten per cent, with one per cent thiourea or ten per cent stannous chloride, all of which inhibit oxidation

The ... not specific as sulphydryl groups ... urinary ... atropine, iron and reductones interfere Reductones are produced from hexoses when foods are heated in alkaline solution and reduce the blue dye in a manner indistinguishable from that of ascorbic acid Dehydroascorbic acid which functions in the body as ascorbic acid, is not estimated by indophenol and must first be converted into ascorbic acid by hydrogen sulphide The interference from reductones is largely eliminated by titration in the presence of formaldehyde or by the method of Harris and Mapson [10] which depends on the principle of the continuous flow, by which the complete time course of a reaction can be followed At pH 3 5 and 20° C after 0 28 0 55 0 75 and 1 1 seconds respectively, sixty four, eighty, eighty four and eighty nine per cent of the reaction with ascorbic acid is complete whereas other values are obtained with other reducing substances By plotting curves of reaction times both ascorbic acid and interfering substances can be estimated when present toget... lene is another interfering ... fluids interfere with the tit... by using a photo electric colorimeter or by electrometric titration ... possible method of separating ascorbic acid from interfering substances is by paper partition chromatography using phenol acetic acid as a solvent [12] The method of Roe and Kuether [360] depending on the reaction between 2 4 dinitrophenylhydrazine and dehydroascorbic acid to form a red compound which can be estir... ...oids the interfering substances pre... by Roe and his colleagues [15] to ... dehydro

ascorbic acid and diketogulonic acid, which was formerly estimated as ... n the presence of one another Diketogulonic acid has

high values if not allowed for

its ability to decolorize methylene

blue [583, 584] It ... blood and urine

using the reagent

to produce a color

only interfering substances being reductones, reductic acid and cysteine.

Bioassay is rarely used, but *is still the final criterion for judging whether* a preparation has an antiscorbutic action Preparations are assayed by prophylaxis or cure of scurvy in guinea-pigs, from the rate of growth of the lower *incisors of the guinea-pig*, from the length of the odontoblasts, and from growth response [20] The increase in serum alkaline phosphatase in scorbutic guinea pigs observed after a critical dose of ascorbic acid has also been used [891]

UNITS OF ASCORBIC ACID

Since the availability of pure ascorbic acid the unit based on a biological assay has been unnecessary The International Unit, which was in use before its isolation and synthesis is equivalent to 0 05 mg of ascorbic acid

DISTRIBUTION OF ASCORBIC ACID IN FOODS

Ascorbic acid appears to be present in all living tissues, but fresh fruits and plants are the best

haws black and red cu

(mainly in the peel) F

and melons contain ne

sprouts, kale, broccoli, watercress and turnips (root and tops) contain relatively large amounts Half a pound of potatoes supplies about 30 mg

and autumn, e g spring potatoes only contain 5 to 10 mg of ascorbic acid and autumn tubers 20 to 33 mg Variations may be found in different fruits and leaves on the same plant In fruits the outer portion contains more than the centre, except in potatoes, in which the skin contains less than the rest [901] Dried legumes and cereals are poor sources of ascorbic acid

The richest source of ascorbic acid is rose hips, which should be picked before they are over ripe, otherwise they may lose half their content Rose hips are converted into syrup, which since 1942 has been issued by the Ministry of Food, with a declared potency of 175 to 200 mg per 100 ml It has been shown, however, that such syrup loses its ascorbic acid fairly quickly In three to five months there may be a loss of fifty per cent in the ascorbic acid content, in one case it was ninety per cent [894] A high sugar content and the presence of sulphur dioxide minimizes the destruction of the vitamin in fruit syrups on storage [914] Half a pound of potatoes, a *helping of cabbage or brussels sprouts*, and an ounce of watercress daily supply sufficient ascorbic acid provided the foods are not spoilt in cooking and provided the cooking water is consumed as well Small but appreciable quantities are present in animal products Indeed it is possible to remain free from scurvy on a diet consisting solely of underdone meat (p 439), and Eskimos, who live largely on animal flesh do not suffer from scurvy The richest animal sources of ascorbic acid are glandular tissues, particularly the suprarenals, and actively functioning tissues

The ascorbic acid content of cows milk is very variable The value drops as soon as it leaves the cow and cools down Even in twenty four hours raw milk may lose twenty to thirty per cent Traces of copper in the vessels sunlight and pasteurization reduce the quantity still further [34] Unless special care is taken pasteurization may result in the loss of thirty to sixty per cent of the ascorbic acid Even after the delivery of domestic milk the small residual amount of ascorbic acid is further decreased by keeping on the doorstep (p 396) and by reheating [832] Exposure to ultra violet light on the doorstep causes loss of ascorbic acid A pint of commercial milk contains from 4 to 13 mg the same amount of mother s milk contains 14 to 30 mg in this country

Effect of Cooking When ascorbic acid is heated in solution with alkalis it is rapidly inactivated In natural foodstuffs there are stabilizing factors such as organic acids (tartaric citric) and sugars (fructose sucrose glucose) preventing its destruction During cooking a proportion of the ascorbic acid is extracted and passes into the cooking water from twenty five to sixty per cent may be lost in this way if the cooking water is not consumed As soon as vegetables or fruits are gathered an enzyme known as ascorbic acid oxidase is liberated and this slowly *oxidizes ascorbic acid. It is formed* when fruits or vegetables are bruised macerated or steeped in water Ascorbic acid oxidase is a specific copper protein blue green in colour with a molecular weight of 150 000 and containing six copper atoms per molecule [18] Its activity does not depend upon its copper content the copper being non ionizable [19 23] Fruit and vegetables should therefore not be kept too long before cooking Cooking in iron copper or badly tinned vessels causes destruction of ascorbic acid vessels of alkali free glass (pyrex) stainless steel aluminium or enamel have no *deleterious effect Boiling fruits and* vegetables with the lid on the vessel for the minimum period of time in the smallest quantity of water conserves the maximum quantity of the vitamin [883] The material should be plunged straight into boiling water to destroy the ascorbic acid oxidase as soon as possible and cooked with the vessel lid on The rate of boiling is important on account of the ratio of water to vegetables at the end of cooking The loss occurring on cooking fruit and vegetables varies enormously [illegible] may vary *from twenty seven to seventy* [illegible] occur on rejecting the cooking water [illegible] destruction of ascorbic acid particularly if soda is added for cooking purposes Soda however may diminish the cooking time of vegetables such as peas and thereby conserve ascorbic acid

A considerable amount of ascorbic acid is retained in jam making about thirty to forty per cent of that present in the original fruit escaping destruction the sugar and the sulphur dioxide used for preserving fruit pulp *protect against oxidation* [914] The content slowly declines on keeping e g twenty to thirty per cent after three months [902] although the figure is very variable and the loss may be as little as ten per cent after six months keeping Black currant syrup keeps well even after a year s *storage* The content is affected by exposure to light air and elevated temperatures [899]

Boiling conserves more ascorbic acid than steaming but in the latter process there is practically no waste in the cooking water which usually goes down the kitchen sink and may extract as much as sixty per cent of the ascorbic acid present in the food As served steamed *green* vegetables actually contain twenty five per cent more ascorbic acid than when boiled Baked potatoes contain approximately as *much ascorbic* acid as boiled and weight for weight chips contain more as much water is driven off in frying [896] Mashing or whipping up potatoes and keeping them hot destroys their ascorbic acid rapidly Most of the vitamin is preserved *if the potato* is cooked and served in its skin [882] Chopping vegetables before or after

cooking destroys a considerable amount of ascorbic acid particularly if a steel or metal knife or chopper is used [21] a shredded lettuce loses eighty per cent in one minute [22]

oking and hence more ascorbic
acı tew pot kept going for hours is
cer acid Frying being a quick
method does not cause very much destruction of ascorbic acid provided the temperature is not too high [896]

Pressure cooking if timed correctly does not result in any more destruction of ascorbic acid than other methods of cooking [1023] the average is twenty two to twenty eight per cent [30] It may result in less destruction because practically no water is used and therefore little is leached out [27 28] If pressure cookers are misused considerable destruction of vitamins may occur Waterless cooking results in less loss of ascorbic acid than any other method about seventy three per cent is retained [30]

Radio frequency or high frequency heating which has attained an important position in the food industry in America causes no more destruction of ascorbic acid in foods than ordinary methods of cooking [74]

Restaurant cafeteria and Army meals usually contain little ascorbic acid because of the long time elapsing from the peeling and preparation of the vegetables to their appearance on the plate [898 900] The hot plate and steam table cause rapid inactivation Olliver [896] has shown that keeping cooked cabbage hot for fifteen minutes results in a twenty five per cent loss of ascorbic acid this is increased to seventy five per cent after ninety minutes

Effect of Storing Cold Storage Canning Dehydration Preserving etc Loss of ascorbic acid may occur if fresh foods are stored for any length of time between purchase and consumption [25] According to Olliver [29] there is little loss in cabbage stored for a week under normal conditions although other authorities state that there is a drop of fifty to eighty per cent after two to four days storage [25] Temperature freshness care in handling exposure to the sun and strong wind are important factors A loss of ten per cent for every day s storage has been reported for green vegetables and bruised soft fruits [896] Root crops keep better because there is less surface and they are protected by a tougher epidermis although losses may be considerable if they are stored for months Fruit and vegetables should not be cut up and left for hours before consumption

There is no appreciable loss of ascorbic acid in fruits and vegetables kept in cold storage or treated by the quick freeze process [26 885] Generally speaking frozen foods contain more than canned foods [51] Some losses do

domestic refrigerators for a few days Little loss occurs during defrosting after freezing and after cooking the defrosted food much ascorbic acid is retained e g up to eighty per cent [904] peas lose forty to fifty per cent [903] Even if kept cold or refrigerated after cooking cooked food rapidly loses its ascorbic acid

Pickling curing and salting result in complete destruction of ascorbic acid The vitamin is largely retained in pulped fruit if this is treated with sulphur dioxide e g sulphited black currants retain sixty three per cent of their ascorbic acid for sixteen months [36]

Canned foods are excellent sources of ascorbic acid about sixty seven to ninety three per cent being retained in the process [33 35 886] If the food

ost [35] although
beans [37] The
A good vacuum
A considerable

amount (about fifty per cent) is present in the canning liquid Cold canned foods usually contain more ascorbic acid than foods cooked in the household Storage in the can results in a slow loss of ascorbic acid , this can be minimized by storage in a refrigerator [886] Contamination with copper of the can destroys ascorbic acid rapidly , this has been overcome by suitably lining the can Once the can is opened the vitamin is fairly stable for a few days if the food is kept in a refrigerator

Dehydrated vegetables have recently been popularized The degree of ascorbic acid retention depends on the method of preparation Sun dried foods contain little or none The material should be cooked or scalded rapidly before dehydration to inactivate ascorbic acid oxidase sulphite or dehydration in the absence of air retards the oxidation of ascorbic acid According to Olliver [29] about sixty per cent is lost in the factory dehydra tion of cabbage On reconstitution dehydrated cabbage only recovers about fifty per cent of the original moisture content, and during domestic cooking on an average about thirty per cent is retained in the cooked food About fifty per cent is extracted, and the remaining twenty per cent is destroyed [29] This compares favourably with the cooking of fresh cabbage From eleven to fifty six per cent of the ascorbic acid is lost in the dehydration of potatoes [906] Further loss occurs on storing e g fifty per cent in three months Dehydrated vegetables are best cooked by placing directly into boiling water and cooking for twenty minutes and not by preliminary soaking in cold water which leaches out some fifty per cent of the ascorbic acid [897 910]

Considerable amounts of ascorbic acid are retained in fruit preserved by domestic processes [30] Black currants and gooseberries preserved as whole fruit by heat processing methods in glass or metal containers retain sixty one and sixty eight per cent after eighteen months storage , raspberries retain forty seven per cent , and strawberries twenty five per cent The use of plastic seals for covering heat processed jars of fruit leads to considerable loss of ascorbic acid e g eighty per cent [86]

Spray drying of liquids such as milk results in a loss of about twenty per cent of the ascorbic acid , roller drying causes a loss of thirty per cent and evaporation about sixty per cent

Ultra violet light inactivates ascorbic acid [832] This is important if jams preserves etc are kept for weeks or even months in shop windows Such preparations should be marketed in tins or in amber coloured glass The conditions are not the same as in fresh foodstuffs exposed to solar ripening when enzymes pigments and organic acids protect the ascorbic acid from inactivation Fifty per cent of the ascorbic acid in milk may be destroyed in one hour if it is left in a glass bottle on the doorstep in bright sunlight [832]

Ascorbic Acid Content of Foods The ascorbic acid content of various foods before and after cooking and canning is given in the following table

Ascorbic Acid Content of Foods

Foodstuff	Description	Ascorbic Acid in mg per 100 grams or 100 ml (3½ oz)	Remarks
Fruits			
Apple	—	8 22	Av 8
	Cooked	4	
	Skin	6 1 15 5	
	Jam and jelly	2	
	Bramley seedling	16 22	
	peel	83	
	dried	11	
	Cox s orange pippin	14 4	

Foodstuff	Description	Ascorbic Acid in mg per 100 grams or 100 ml (3½ oz)	Remarks
Fruits—continued Apricot	Fresh	4 11	
	Canned	5	80 100% retained
	Dried	1–2	
	Jam	2 12	
Avocardo	—	10 16	
Banana	Ripe	10	
Barberry	Raw	81	
Bilberry	Cooked	7	
Blackberries	Raw	10	
	Cooked	8	20 when raw
	Jam	5	
	Jelly	2	
lueberry	Raw	4–75	Av 10
ierry	Raw	2 8 10	
	Cooked	3	
inberry	Juice	15	
rrant black	Raw	108–419	Av 150
	Juice	90 360	
	Cooked or canned	90	
	Jam	50	
	Juice	140	
	Puree	70	Ministry of Food
red	Raw	30–45	
	Cooked	23	
	Jam	6	
white	—	23	
Custard apple	Raw	1 3 15	
Damson	Cooked	3	
Elderberry	Raw	8–10	
Fig	Fresh	2 2–8 72	
	Dried	0	
Gooseberry	Fresh	25–40	
	Cooked	20	40 mg when raw
	Jam	11	40 mg when raw
Grape	Raw	4	
Grapefruit	Juice	37 50	
	Oil gland layer	314	
	Mesocarp	210	
	Endocarp	76 6	
	Canned	41	90 100% retained
	Canned sweetened	45 50	
	Ditto after 6 months	39–46	
	,, ,, 12 ,,	34–41	
	Marmalade	5 5	
Greengage	Raw	5 0 6 5	
	Cooked	2 7 5 7	5 0–6 5 mg when raw
	Jam	2	
Guava	—	75	
,, (S African)	—	250	
Haw	Raw	40–500	
Hip rose	Fresh	6~ up to 1 800	
	Brought to boil	394	418 mg when raw
	Boiled 5 min with sugar	238	,,
	,, 10 ,,	216	,,
	Jelly	100	
	Syrup	175 200	Ministry of

Foodstuff	Description	Ascorbic Acid in mg per 100 grams or 100 ml (3½ oz)	Remarks
Fruits—continued			
Huckleberry	—	30	
Lemon	Juice	30 78	Av 45
	Pulp	14–16	
	Peel	100 205	
	Marmalade	10	42 mg when raw
Lime	Pulp	20–60	
	Juice	16 8 62 5	Av 37
Litchi	—	2 20	
Loganberry	Raw	35	
	Boiled	22 26 7	38 8–48 8 mg when raw
	Canned	31–35	
Mango	Raw	25	
Medlar	Raw	2 0	
Melon	Raw	3 21	
	Cantaloupe	30–42	
	Water melon	1 0 7	
Mulberry	Raw	6 6–21	
Nectarine	Raw	8 24	
Olive	Raw	15	
Orange	Pulp	16–47	
	California (juice)	52	
	Brazil (pulp)	34–62	
	Jaffa	33 54	
	Navel	52 98	
	Juice	22–89	Av 58
	Peel	75 8 210	
	Canned	94–100% retained	
	Marmalade	7 14	50 mg when raw
Papaya (pawpaw)	Raw	36 115	Av 45
	Skin	116	
Peach	Raw	2 17	Av 10
	Canned	60 90% retained	
Pear	Raw	3 7	
	Canned	1 5	
	Cooked	3	
Peppers	Green	125 180	
	Red ripe	150	
Persimmon	—	100	
Pineapple	Raw	3 13	
	Canned	8	
Plum	Fresh	3 7	
	Dried prune	1 0 2 0	
	Boiled	2 2 2 9	4 6 mg when raw
	Canned	2 2 2 5	
Pomegranate	Raw	6–15 6	
Pumpkin	Raw	5	
	Cooked	2	
Quince	Raw	9 12	
Raspberry	Raw	19 37	Av 30
	Canned	3 9 8	
	Jam	8	
Rowan (mountain ash)	—	35 50	
Strawberry	Raw	46 234	Av range 50 80
	Boiled	25 37	71 4 mg when raw
	Canned	21 35 7	

Food stuff	Description	Ascorbic Acid in mg per 100 grams or 100 ml (3½ oz)	Remarks
Fruits—continued			
Tangerine	Pulp	10–36	
	Juice	10 78	
Whortleberry	—	5	
Nuts			
Almond	—	<19 3	
Cashew	—	180	
Chestnut	—	6	
Cob	—	3 15	
Coconut	—	0 4 13 4	
Hazel	—	2 74 15	
Peanut	—	10	
Pecan	—	2	
Walnut	Unripe	160	
	Chutney (home made)	98% of original	
	Chutney (commercial)	40% of original	
Vegetables			
Artichoke globe	Raw	9	
	Cooked	6	
Jerusalem	—	7	
Asparagus	Whole	35	
	Canned	80 100% retained	
	Cooked	30	
Bean broad	Raw	27 7 37	
	Boiled	10	
	Canned	14 7 17 6	
Bean green snap or string	Raw	19 25	
	Canned	40 75% retained	
	Cooked	7 2 20 2	
	Dried	0	
Bean soya	Black dried	30–40	
	Green dried	17 75	
Beetroot	Root	15	
	Cooked	5	
	Canned	2	
	Tops	34–50	
Broccoli	Entire plant	70 110	
	Boiled leaves	22	32 mg when raw
	Dehydrated	6 0	
Cabbage	Raw	60 118	Av 70
		20–43% retained	
	Cooked	11 57 70	
	Fresh boiled 10 mins	49	
	15	29	
	30	22	
	60	15	
	90	5	
	Dehydrated (uncooked)	220 376	30–80% retained Av 60–70%
	(cooked)	42 62 190	
Carrot	Raw	5 10	
	Cooked in skin	90% retained	
	Shredded	80% retained	
	Canned	2	
Cauliflower	Raw	69 75	
	Cooked	37–40	

Foodstuff	Description	Ascorbic Acid in mg per 100 grams or 100 ml (3½ oz)	Remarks
Vegetables—contd			
Cauliflower—*cont*	Dehydrated (uncooked)	290	
	,, (cooked)	17 60	
Celery	Stalks	5 7	
Chard	—	35–42	
Chives	—	70 119	
Corn (sweet)	—	10	Av 6 12
Cucumber	—	8	
Dandelion	Leaf	100	
Endive	Unblanched	15 24	
	Blanched	4	
Garlic	—	14	
Grass	Fresh	68 , 75 3	
Horseradish	—	90	
Kale	Raw	100 150	
	Boiled	23–40	
	Dehydrated	170 295	
Kohlrabi	Raw	60 117	
Leek	Raw	15 20	
	Cooked	10 15	
Lettuce	—	8–18	
Lucerne (alfalfa)	Fresh	73 380	
Mango	—	25 60	
Marrow	Raw	11	
	Cooked	2	
Mint	—	39	
Mushroom	—	3	
Mustard and cress	—	37	
	Seeds	44	
Nasturtium	Leaves	200–465	
Nettle	Leaves	50 , 72	
Onion	Bulb, raw	15 30	
	Spring	25	
	Cooked	6	
	Dehydrated	37	
Parsley	Leaves	154–209	
Parsnip	Raw	18 30	
	Cooked	4–10	
Pea	Fresh green	25	
	Dried	0	
	Boiled	14–16	
	Quick freeze	12 23	
	Canned	10	45 90% retained
	Split	2	
Pepper	Green	120 180	
	Red	150	
Potato	Tubers raw	11 36	Av 18
	New	20–33	
	Old	5 10	
	Stored 180 days	10	18 mg raw
	Baked	7	,,
	Peeled and raw	19	
	,, boiled	52% retained	
	,, sliced and boiled	47% retained	
	Boiled whole	60% retained	
	,, cold	2 6	18 mg raw
	,, unpared and mashed	11 31	87% retained
	,, and creamed	2	18 mg raw
	,, fried	2 1	,,
	Chips	60% retained	

Food tuff	De cripti n	A corbic Acid in mg per 100 gran s or 100 ml (3½ oz)	Rema ks
Vegetables—contd			
Potato—contd	Dehydrated	16 25	60% retained
	reconstituted	6	55% retained
Potato sweet	—	22–33	
	Boiled whole	100% retained	
Pumpkin	Cooked	2	
Radish	Root	29 36	
Rhubarb	Fresh	20 25	
	Cooked	4	
	Jam	2	
Shallot	Bulb	6	
Spinach	Leaves	50 80	
	Boiled	15 71	
	Quick freeze	32	
Sprouts	Fresh	65 150	
	Boiled	43–85	
	Quick freeze	51	
Squash	Raw	29	
	Cooked	7 17	
Swede	Root raw	44	
	cooked	2	
Tomato	Raw	10–38	
	Canned	14–21	17 5 mg (U S A)
	Juice	16–33	
	Late	5 8	
	Canned after 6 months	7 18	
Turnip	Root	30	
	Cooked	13 15	50 60% retained
	Tops	100 140	
	Boiled	18	35 mg when fresh
		17% destroyed	
		28% diffused	
Watercress	—	61 89	
Dairy Products			
Milk	Cow s raw	0 5 2 96	Av 2 2 mg
	colostrum	1 77	
	boiled	0 4	0 7 mg raw
	pasteurized	0 3 5 8	Av 1 3 mg
	evaporated	0 4–2 76	
	skimmed dried	1 58–6 27	
	whole dried by roller process	6 9 9 7	12 3 per reconstituted litre
	Goat s raw	0 5 2 0	
	boiled	0 4	0 9 mg fresh
	Human	1 2 10 8	Av 5 1 (U S A) 3 2 3 8 (U K)
		8 10	well fed mothers
		1 2–4 0	poorly fed mothers
		7 0	colostrum 10 days post partum
	milk depots	1 31±0 2	
Cheese	—	nil	
Eggs hen s	—	nil	
duck s	—	0 3 1 3	
Fish			
Carp			
	Liver	4 5–11 3	
	Muscle	0 5 1 88	

Foodstuff	Description	Ascorbic Acid in mg per 100 grams or 100 ml (3½ oz.)	Remarks
Fish—continued			
Clam	—	30	
Cod	Liver	26.7	
	Roe	120–160	
Crab	Liver	27	
	Muscle	13	
Eel	Liver	9.8–11	
	Muscle	1.4	
Haddock	Roe	10	
Herring	Roe	20	
Lobster	Liver	24	
	Muscle	5	
Mackerel	Roe	40	
Mussel	Liver	30	
	Muscle	3	
Oyster	Liver	12	
	Muscle	3	
Salmon	Whole fish	89–215	
	Chilled	9	
	Canned	0	
	Roe	14	
Scallop	Liver	11.5	
	Muscle	3	
Meat and Poultry			
Calf	Liver	30–50	
	Muscle	7.8	
Duck	Liver	13–68	
	Muscle	7.8	
	Heart	24.2	
Fowl	Muscle	1.5–6.0	
	Liver	28–43	
	Brain	11.4–26	
	Heart	3.8–4.0	
	Kidney	10.8	
	Liver	35	
	Muscle	1.6–2.2	
Ox	Liver	27–40	
	Muscle	1.7	
Pig	Brain	25	
	Kidney	14	
	Liver	11–41	
	Muscle	1.9	
Rabbit	Muscle	0.42–3.4	
Sheep	Brain	15.4	
	Heart	6.2	
	Liver	25–50	
	Kidney	10.9	
	Muscle	2.5	
Miscellaneous			
Beer	—	nil	
Corn		2.1	
Cider	—	trace	
Coffee	Freshly ground	56–61	
Honey	—	0–20	
Rice	—	2.4	
Tea	Fresh leaves	120	
Wheat	—	2.6	
Yeast	Baker's	1.6	

What is perhaps more important than the ascorbic acid content of a foodstuff when raw or cooked by a special process is the content of the food as served at the table. Fincke and her colleagues [53] calculated the ascorbic acid content of an average helping of food as served on the plate in American college dining halls. Their figures are given below.

Ascorbic Acid Content of Food as Served [53]

Food	Mean of all Values Obtained in Four College Dining Halls (mg. per portion served)
Apple, raw	8
baked	4
rings	1.0
sauce	0.7
Apricots	7.1
Asparagus	8.4
Beans, baked	2.1
green	1.1
Beef, roast	2.2
Beets	4.9
Broccoli	27.9
Brussels sprouts	24.3
Cabbage, cooked	11.1
Carrots, raw	2.1
cooked	1.6
and celery, cooked	2.2
and peas, cooked	0.7
Cauliflower	28.4
Celery, raw	1.6
Cherries	4.5
Chicken	2.2
Chili	10.1
Corn	4.2
Cucumber	1.3
Custard	1.2
banana	3.1
Egg, cooked	1.8
Farina	1.0
Figs	1.1
Fruit, mixed	9.2
juice, mixed	4.6
Goulash	7.4
Grapefruit	48.1
juice	45.2
Ham	1.4
loaf	3.9
Hash	1.2
Jam, jelly, honey and marmalade	1.0
Ketchup	3.6
Lemon	4.3
Lemonade	8.7
Liver	7.1
sausage	1.2
Meat loaf	2.5
pie	2.5
sandwich	0.6

Food	Mean of all Values Obtained in Four College Dining Halls (mg per portion served)
Milk	1·6
Olives	1·2
Onions, raw	2·7
,, boiled	3·3
Orange	59·8
Parsnips	2·8
Peaches, canned or dried	3·4
Pears, canned	0·5
Peas	4·5
,, and turnips	2·6
Pickles	0·7
Pie, apple	0·4
,, pumpkin	—
Pineapple juice	28·3
Plums, canned	1·3
Pork chop	2·1
,, roast	1·1
,, sausage	1·3
Potato chips	4·0
Potatoes, baked	10·5
,, boiled	6·1
,, creamed	4·1
,, fried	1·9
,, mashed	2·5
Prunes	6·6
Radishes	3·2
Rhubarb	2·7
Salad, apple	4·7
,, ,, and carrot	5·1
,, ,, ,, nut	2·0
,, ,, ,, orange	20·0
,, ,, ,, raisin	1·7
,, banana	1·7
,, ,, and pineapple	6·4
,, cabbage	26·3
,, ,, and celery	9·8
,, ,, ,, raisin	25·4
,, ,, ,, tomato	19·3
,, carrot	3·7
,, ,, and celery	3·7
,, ,, ,, pineapple	1·1
,, ,, ,, radish	5·1
,, ,, ,, raisin	3·3
,, cottage cheese	1·1
,, dressing, French	0·8
,, fruit	9·3
,, ,, in gelatin	1·0
,, grapefruit	14·1
,, ,, and orange	26·6
,, green pepper	4·8
,, lettuce	1·6
,, macaroni	1·2
,, mixed	1·9
,, potato	1·0

Food	Mean of all Values Obtained in Four College Dining Halls (mg per portion served)
Salad radish	1 0
tomato	4 0
" " aspic	0 4
tuna fish	1 1
vegetable	11 6
Sauerkraut	4 0
Soup	3 2
bean and pea	12 2
cream of tomato	4 6
pea (split)	0 8
potato	1 8
tomato	5 0
vegetable	6 1
" chowder	7 7
Spaghetti and tomato	3 8
Spinach	9 4
Squash	6 1
Stew	3 9
Strawberries	61 6
Stuffing	2 0
Sweet potato	12 0
Tomato juice	5 5
sauce	18 9
Tomatoes sliced	24 0
stewed	2 9
Turnips	10 8
Veal	4 1
Vegetables mixed	2 9
Watercress	10 0

PHYSIOLOGY AND FUNCTIONS OF ASCORBIC ACID

Ascorbic acid is synthesized by all the higher plants probably by simple organisms such as moulds and bacteria and by many animals except the guinea pig and the primates. In the rat which synthesizes its ascorbic acid, radio active tracer studies have shown that glucose is the source of ascorbic acid [209] It is formed during the germination of seeds

The role of ascorbic acid as an activator for the growth of tissues is well recognized It stimulates the growth of fowl monocytes [464] and neoplastic tissue e g sarcoma cells [465] It has a beneficial effect on the formation of fibres in fibroblasts in tissue culture [657] Epithelial sheets of tissue will not grow in media devoid of ascorbic acid [955] and young actively growing cells show a high concentration of the vitamin [340]

Collagen Formation Ascorbic acid is essential for the formation of inter cellular substance in the animal organisms [39 40] Wolbach and his associates [41 44] in a series of papers from 1926 to 1937 showed that reticulum and collagen are not formed in the scorbutic animal Normally in the inter cellular substance fibroblasts lie in an amorphous ground substance within which fibrils of a reticulum are found as wavy bands of collagen The fibrils are cemented together by a translucent matrix In the scorbutic animal the ground substance and fibroblasts are present but no collagen is formed After the administration of ascorbic acid bundles of collagenous material are

formed within twenty four hours The role of ascorbic acid as a prerequisite for the formation of intercellular material in the human subject has been established by the work of Hunt [77] and Crandon [68] (pp 413 485)

Once collagen is formed ascorbic acid is not essential for its maintenance [46 1033], it is only needed for the replacement of that destroyed by wear and tear [1033] Hyaluronic acid a *component of connective* tissue ground substance is not formed in the scorbutic state [54] Ascorbic acid does not serve as a precursor or component of collagen this has been shown in *metabolism experiments using ascorbic acid containing* radio active carbon C^{14} [55]

Although the exact mode of action in collagen *formation is not known* recent work with cortisone has thrown some light on the subject It has been shown that adrenocorticotrophic hormone (A C T H) and cortisone can prevent or cure lesions of experimental and clinical collagenous diseases Ascorbic acid is known to be directly implicated in the activity of the adrenal cortex (p 428) so that it seems possible that some of the mesenchymal changes in scurvy are not due directly to lack of ascorbic acid but to hypo *secretion of the adrenal cortex steroids* Cortisone inhibits many of the manifestations of scurvy [56] and it is an attractive hypothesis to suppose that deficiency of ascorbic acid leads to insufficiency of the *glyco* cortico steroids of the adrenal, lack of which may result in defective collagen formation

Structure of Teeth In the guinea pig deficiency of ascorbic acid produces lesions in the tooth pulp and dentine [47] changes occur in the odontoblasts which become shorter and spindle shaped and finally produce a substance resembling bone which fills the pulp canal The dentine becomes porotic and resorbed and *instead of new dentine a spongy porous bonelike material* is laid down The pulp tissue and newly formed bone become atrophied and resorbed with the formation of small cysts Alveolar resorption in the jaw occurs and extends to the outer edges of the mandible [60] If the deficiency of ascorbic acid is absolute the pulp is shrunken and completely detached from the dentine [41, 42] and the enamel is hypoplastic [49] Fish and Harris [48] noted changes in the enamel and cement of scorbutic guinea pigs Boyle Bessey and Wolbach [43 44] observed the effect of ascorbic acid deficiency on the parodontal structures of guinea pigs The teeth became loosened *alveolar bone underwent rarefaction* and periodontal membrane widened Resorption of alveolar bone and cementum with loss of collagenous fibres of the periodontal membrane and depletion of the hemopoietic cells of the bone marrow were obs T the teeth could be easily removed and of the periodontal r

According to Wilton and Thorell [65] ascorbic acid deficiency preve maturation of *young dentine cells and causes reversion of mature cells* to a state resembling immature cells

There is no evidence that in man dental decay is associated with deficiency of ascorbic acid [916]

According to Roff and Glazebrook [57] parodontal disease in man may be due to a deficiency of ascorbic acid (p 463)

Bone Formation and Repair Ascorbic acid is also essential for the formation of bone and cartilage (*see Fig* 127) The gross and histological changes seen in the bones of scorbutic animals are similar to those seen in other structures The lesions are c the costochondral junction the distal end of the femur the the tibia femur and wrist In the affected b one formation the existing osseous shell becomes raref increased t racture on slight trauma particularly a sial junctic bones d false motion at the costoc ons Th lt to form intercellular ma bone teristic

shape and migrate from the trabeculæ to the diaphysis Formation of cartilage and bone matrices ceases The osteoblasts become surrounded by

œdematous connective tissue at

rarefaction of the cortex is

observed bone ceases to grow and the normal junction is replaced by a zone of connective tissue poor in collagen and in which are embedded fragments of calcified cartilaginous matrix devoid of osteoid tissue Controlled experiments show that the connective tissue cells of the marrow are osteo

ists Ascorbic acid appears

ie osteoblasts, which in its

to form a fibrous union

he periosteum

from the bone

cortex occurs Kodicek and vergrowth of subperiosteal bone and porosis in animals suffering from prolonged ascorbic acid deficiency the overgrowth is trabecular not compact bone and is

state (Fig 170) The response to treatment with ascorbic acid is dramatic Within twenty four hours new intercellular substance is formed the fibroblasts are surrounded by a thin shell of osteoid material trabeculæ are formed proliferation of osteoblasts in the periosteum ceases and hæmorrhage from the fragile capillaries stops Capillary formation which is essential in growing tissue is resumed

Mouriquand [61 63] has described the bony defects resulting from acute and chronic ascorbic acid deficiency In acute deficiency softening and rarefaction of the bone occurs particularly in the region of the femoral or tibial epiphyses In chronic deficiency he describes a syndrome resembling chronic osteo arthritis with osteophytic outgrowths pseudoankylosis decalcification of the epiphyses and diaphysis and periosteal thickening

stiffening of the joints in scorbutic guinea pigs

The deposition of bone salt in both normal and regenerating bone is retarded in scorbutic animals but not in the bone of animals receiving adequate ascorbic acid A deficiency of ascorbic acid lowers the alkaline phosphatase of both bone and blood [880] and Bourne [87] has demonstrated that there is less phosphatase activity in regenerating bone from scorbutic animals than that from normal guinea pigs Follis [78] has shown that in the epiphyseal cartilage of scorbutic guinea pigs there is no cytochrome oxidase and the cells contain no glycogen no mucopolysaccharide and a reduction in ribose nucleic acid This is in contrast to the findings in normal bone Follis [917] believes that some of the changes in cartilage seen in scurvy result from a combination of non specific inanition and mechanical disturbances resulting from the absence of osteoblastic activity in the shaft beneath the cartilage

Ascorbic acid is essential for the formation of callus in the union of fractured bones Fractures heal badly in a subject human or animal deficient in ascorbic acid Wolbach and Howe [41 42] have demonstrated the complete failure of natural callus formation in experimental scurvy and the resorption of old callus and the refracture of united fragments of bone has been described in scurvy since early times Callus is normally consolidated into compact bone by thickening of the trabeculæ in the scorbutic state this does not occur [52] It has also been shown that the excretion of ascorbic acid falls rapidly in an animal with multiple experimental fractures [58] and

ASCORBIC ACID AND WOUND HEALING

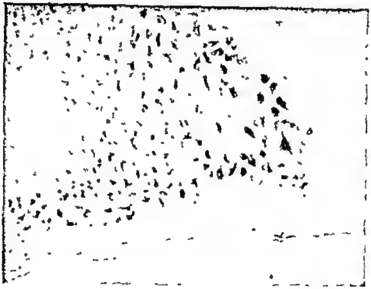


FIG. 127. Growing point of cartilage in a chick embryo, showing cartilage cells laden with ascorbic acid granules. Silver stain ($\times$ 300)

FIG. 128. Ascorbic acid and Wound Healing Section of wound in skin of cat after seven days. In the scar tissue are numerous histiocyte like cells *containing granules of ascorbic* acid. Silver stain ($\times$ 300)

Control.

Subscorbutic.

FIGS. 129 and 130 Ascorbic Acid and Wound Healing. Abdominal incisions of guinea-pigs twenty-one days after operation (life size) Both healed by first intention. *Left,* control animal receiving adequate ascorbic acid, *right,* subscorbutic animal In the control the scar is practically invisible In the subscorbutic animal it is puckered, stretched, sunken and shows a mauve discoloration.

ASCORBIC ACID AND WOUND HEALING

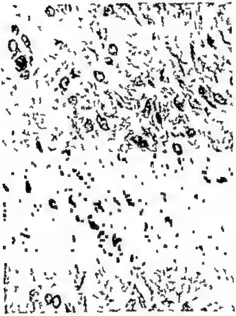


FIG. 131. Control.

FIG. 132. Subscorbutic.

FIG. 133 Control

FIG 134. Subscorbutic

FIGS [illegible] Sections from the abdo-
[illegible]r operation (× 300)
[illegible]igs 132 and 134 from
Fig. 132 fibroblasts,
[illegible]ere is no van Gieson
staining intercellular substance in the scar of the subscorbutic animal

with silver.

that the degree of healing in bone is proportional to the ascorbic ac Bourne [920] has shown that the optimum formation of bony tra the injured femora of guinea pigs is brought about by the adminis 2 mg of ascorbic acid daily and that anything less than 1 mg retards the formation of bony trabecul e Provided the level of acid in the tissues is optimal additional vitamin does not acceler regeneration This was shown by Bourne [921] in the case of rats synthesize their own ascorbic acid

Fractures are *sometimes associated with damaged muscle fibres* scorbutic animal more of the damaged fibres degenerate than ma the muscle tissue so lost is replaced by large masses of hyperpl tissue which is avascular or nearly so [52] In the scorbutic a capillary formation in the region of a fracture can be demon the administration of ascorbic acid, capillary proliferatio and hyperæmia occur [59]

It has long been known that degeneration of striated muscle oc

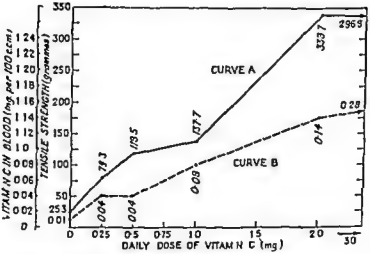


FIG 135 *Tensile Strength of Wounds and Ascorbic Acid Intake* Curve A tensile strength of wounds in fifty two ... intakes of ascorbic acid the tensile strength of ...

A maximum

scurvy hyaline degeneration and fragmentation of the fibres are describ Boyle and Irving [1028] noted hyaline changes only in chronic scurvy acute scurvy myofibrils became detached from the sarcolemma

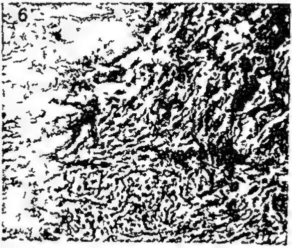
Wound Repair *Clinical and laboratory observations have shown* co clusively that the rate and efficiency of primary wound healing depend on t ascorbic acid and protein concentration in *the tissues* By special hist chemical staining techniques with silver nitrate due to Bourne [70] Goug [387] and Giroud and Leblond [71] it has been shown that appreciable qua tities of ascorbic acid are mobilized from *the tissues* and concentrated healing wounds and young granulation tissue [69] At the same time there a diminished excretion of the vitamin in the *urine* [923] The lowest excretio occurs six to nine days after wounding and precedes the period of most activ collagen formation The tensile strength of scorbutic guinea pig wounds considerably less than normal [72 73 925] The tensile strength of the wour is in fact proportional to the ascorbic a content of the wound and of th blood [924 926] After surgical op e tensile strength of wounds i the human is decreased if the pl c acid falls below 0 2 mg p ml [852] In t a da 2 mg is adequate for optim

ASCORBIC ACID AND WOUND HEALING

Fig 136 Unorganized blood clot in wound of a guinea pig receiving no ascorbic acid Masson trichrome stain (× 100) Section made after a week's healing Tensile strength of wound 46 grams

Fi[illegible]

there is a large empty space beneath the epithelial layer Tensile strength of scar 34 grams



[illegible] rs of cells in the scar The epithelium [illegible]overed the scar completely Tensile [illegible]th of scar 62 grams

Fig 139 Reticular preparation made from wound shown in Fig 138 (× 120) On the left is normal connective tissue on the right are numerous reticular fibres and cells in the scar

ASCORBIC ACID AND WOUND HEALING

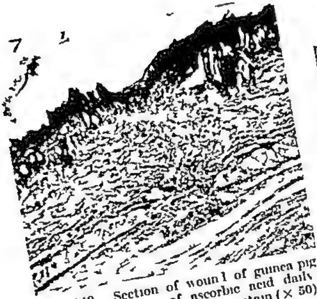


Fig 140 Section of wound of guinea pig receiving 1 mg of ascorbic acid daily Hematoxylin and van Gieson stain (× 50) The scar contained about the same amount of fibrous tissue as the scar in the guinea pigs receiving 2 mg of ascorbic acid (Fig 142 143) but more of the fibres were reticular Tensile strength of scar 158 grams

Fig 141 Reticu... section of which is show... Numerous fine reticular fibres but le... cells are present

Fig 142 Section of wound of guinea pig receiving 2 mg of ascorbic acid daily Hematoxylin and van Gieson stain (× 50) There is abundant collagen in the scar Tensile strength of scar 295 grams

Fig 143 Reticulum preparation of wound section of which is shown in Fig 142 (× 50) Few reticular fibres are present and the intensity of staining of scar tissue is similar to that of normal connective tissue

healing, less than this delays the process although more than this has no significant effect on the rate of healing or on the tensile strength of the wound (Figs 136–143) In the scorbutic guinea pig the wound scar is puckered, stretched sunken loose irregular and almost avascular, and the removal of catgut ligatures either by phagocytosis or by extrusion is delayed [77] Some fibroblastic proliferation occurs in the wound, but the fibroblasts do not form syncytia and there is very little extra cellular material If ascorbic acid is administered normal fibroblasts appear and fine reticular fibres and much extra cellular material are formed The latter consists partly of acid mucopolysaccharides of the hyaluronic acid or chondroitin sulphate type [54] It is possible that epithelial regeneration can occur in the absence of ascorbic acid, it does so in the cornea and the gum periosteum [79] When, however, healing of a wound demands new formation of collagenous tissue a deficiency of ascorbic acid delays epithelialization because of lack of a satisfactory collagenous base for the regenerating epithelium There is diminished phosphatase activity in the wound of a scorbutic animal [80]

In 1940 Crandon [68] proved conclusively that ascorbic acid is necessary for the healing of wounds in man This investigator placed himself on a scorbutic diet for six months and after three months a wound was made in his back and a biopsy specimen removed This showed good wound healing, with ample intercellular substance and capillary formation (Fig 144) Another wound was made after six months when the subject was scorbutic and with a zero blood ascorbic acid Ten days after the second wound was made a biopsy specimen showed that beneath the skin which appeared to be well healed there was only blood clot and no evidence of healing it was in fact necessary to insert a rubber drain There was lack of intercellular substance and capillary formation (Fig 145) Then 1 000 mg of ascorbic acid was injected intramuscularly and ten days later a further biopsy made of the wound There was now good healing with the formation of ample intercellular substance (Fig 146) As a result of inadequate collagen formation in the subject deficient in ascorbic acid fibroblastic proliferation occurs but there is no intercellular material (Fig 147) and blood vessels do not penetrate the poorly developed granulation tissue Leakage of blood from the fragile capillaries forms hæmatomata, which are not organized or absorbed and the superficial skin scar is split by them or by extravasations of blood Phagocytosis is delayed and the surrounding structures are not incorporated in the scar tissues of the wound Infection occurs more readily than normally in scorbutic wounds, as noted by Farmer [864] who repeated Crandon's observations on human volunteers Hunt [77] from a study of twenty eight surgical cases that came to post mortem considered that the poorest collagen formation occurred in those patients most deficient in ascorbic acid Carney [97] however, failed to observe any relationship between plasma ascorbic acid levels and satisfactory wound healing in military hospitals Wounds most commonly disrupt on or about the tenth day and it is possible that ascorbic acid deficiency is an important cause of wound disruption In spite of a low level of ascorbic acid nutrition at the time of operation normal wound healing results if ascorbic acid is given orally or parenterally post operatively [852] Sprinkling ascorbic acid on the wound of a rat which synthesizes its own ascorbic acid, has no beneficial effect [85]

Wolfer and his co workers [75] made observations similar to those of Crandon on nine human volunteers They concluded that a diet deficient in ascorbic acid diminishes the tensile strength of the wound by fifty per cent from three to five days up to the fourteenth post operative day They consider that when circumstances for healing are not good as in the presence of undue wound tension or diminished blood supply, failure of primary wound healing is far more likely

Abscesses do not heal and
increase of macrophages is scess

FIG 144 Section from wound of a human subject on a scorbutic diet for three months An experimental wound was made and sutured and a biopsy specimen obtained eleven days later Normal healing has occurred (see text, p 413)

FIG. 145 Section from wound of a human subject on a scorbutic diet for six months, showing absence of healing The wound appeared to heal by first intention Ten days later a biopsy specimen was taken As soon as the skin was divided it was found that the tissues under the skin had not healed at all and that the wound contained firm dry blood clot This is shown in Fig 145 by the large empty space beneath the epidermis (see text, p 413)

FIG 146 Same case as Fig 145 after ten days' treatment with 1 gram of ascorbic acid daily Another incision was then made across the same wound that had formerly failed to heal A section of this shown above, shows that normal wound healing has occurred

FIG 147 Section from wound of human

into the surrounding tissues, and the necrotic centres in the abscess are not walled off [928]

Hines and his co workers [890] have shown that ascorbic acid is essential for the regeneration of damaged nerve The tibial nerve of guinea pigs was crushed and the rate of regeneration studied on diets containing graded amounts of ascorbic acid Regeneration was impaired when the ascorbic acid intake fell below an optimal level of 2 5 mg daily It does not follow that ascorbic acid has any specific action on nerve tissue, as any deficiency in collagenous intercellular material in the tissues supporting a nerve would retard its regeneration

Capillary Resistance The hæmorrhagic manifestations of scurvy have been stated to be due to abnormal capillary fragility Morphological changes have not been detected in the c [illegible]
it has not been proved that th [illegible]
cement substance binding the [illegible]
connective tissue and collagen sheath Actually the minute structure of the *capillary wall is not well known*

There are a number of clinical methods employed for the estimation of capillary permeability These are described on p 464 They can be classed as positive or "negative" pressure tests In positive pressure tests the venous return from a limb is dammed back in order to increase the pressure within the capillaries, in negative pressure methods, suction is applied to the skin and this "negative" pressure is transmitted to the underlying capillaries Capillaries which cannot stand the test pressure are ruptured and petechial hæmorrhages result, and the ease with which such petechiæ can be produced is considered as a measure of the strength of the capillary An area is marked off on the skin of a limb, usually the arm the pressure applied under controlled circumstances for a given time and the petechiæ counted under standard illumination with or without the aid of a lens There is no correlation between the positive and negative tests [66] and many authors do not give adequate directions for carrying out their tests There are too many variables for the method to be of any value in the detection of ascorbic acid deficiency even if it could be shown that if all these variables are controlled there is a true correlation between capillary fragility and ascorbic acid deficiency The petechial count depends on the part of the body tested, the temperature, texture and degree of vascularization of the skin age, time of the day and the season of the year [930] False positive results are liable to be obtained during menstruation after hot baths in septic and acute infections (e g diphtheria), in certain blood diseases (anæmia purpura hæmophilia) as a result of drug therapy (arsphenamine and related drugs) and in acute [illegible]

[illegible] system [illegible] Munro Lazarus and Bell [66] have also noted an apparent improvement in capillary strength to occur spontaneously The fact that capillary fragility shows an increase in the spring suggests it may be related to a fall in the ascorbic acid intake that occurs about this time, although the effect of sunlight, [illegible]
cannot be excluded [illegible]
[illegible]
a [illegible] any signs
c [illegible] d subjects
without clinical scurvy do show capillary weakness They also note that there is frequently no marked impairment of capillary strength when human subjects are experimentally deprived of fruit and vegetables for several weeks or months On the other hand, they state that some subjects with weak capillaries may show an improvement in capillary strength after receiving fruit juices Gothlin in 1933 [82, 83] sought to establish a correlation

between capillary fragility and the level of ascorbic acid nutrition but th majority of workers since that date have failed to correlate them [66–68 81 347 553 877 929] According to Lazarus Munro and Bell [67] petechia counts may occur in patients with scurvy but clinical improvement after treatment with ascorbic acid is not correlated with a lowering of the petechia count These workers consider that determination of the capillary fragility is of no diagnostic value in scurvy because of the wide variations in the results obtained

The problem has also been approached from the histological side No important changes in blood vessels have been described which can be attributed to ascorbic acid deficiency Capillary permeability in the scorbutic guinea pig has been studied by Elster and Schack [89] by observing the rate of transport of the dye T 1824 (Evans blue) across the capillaries After administering T 1824 intravenously to scorbutic and normal guinea pigs they found no qualitative or quantitative differences in the tissue distribution of the dye indicating that capillary permeability was unaltered in the scorbutic state

Observations by Lee and Lee [88] suggest that ascorbic acid might be one of the factors essential for the functioning of certain peripheral vasomotor mechanisms Others have noted a greatly diminished vascular response in the perfused hind limbs of scorbutic guinea pigs These findings could be explained by an adrenaline protecting action exerted by ascorbic acid

They have shown that in scurvy there is failure of the contractile mechanism of the small vessels resulting in their dilatation and a sluggish blood flow (Figs 162 165)

Ascorbic Acid and the Hæmopoietic System It is generally considered that ascorbic acid plays a part in erythropoiesis which is usually depressed in scurvy particularly in the first two years of life Anæmia is of regula occurrence in scorbutic guinea pigs blood films from which show sligh poikilocytosis anisocytosis polychromasia and stippling [90] If the diseas is fatal an increased number of reticulocytes appear in the peripheral circula tion just before death This has been observed in human scurvy [94] Th bone marrow is hyperplastic with an increasing number of normoblasts suggesting arrest of maturation The administration of orange juice t ... ces a reticulocytosis within three days followe ... noglobin concentration The anæmia is no ... imal with iron deficiency because it does no occur ... tered with a scorbutic diet nor does iron prevent its development or effect its cure [99]

Although anæmia is frequent in human scurvy its occurrence is inconstant Of forty three patients with severe scurvy seen by Brown [84] nine showed little or no anæmia Mettier Minot and Townsend [86] found that about a third of a large group of scurvy patients suffered from severe anæmia a third were mildly anæmic and the remaining third had slight or no anæmia Anæmia is not a constant finding in infantile scurvy [92] and it does not occur in sub scorbutic ascorbic acid deficiency [870] Crandon [68] who existed on a scorbutic diet for six months showed a slight fall in blood hæmoglobin which was not surprising as 6 litres of blood were withdrawn for laboratory tests but it responded to iron therapy while other symptoms of scurvy persisted In another study of this kind conducted on volunteers on a scorbutic diet calculated to contain 1 mg of ascorbic acid daily there was no sign of anæmia although other symptoms of scurvy were present [108] However these experimentally induced single vitamin deficiencies cannot be compared with a malnutrition syndrome such as scurvy Lozner [110] reported cases of ascorbic acid deficiency that responded to iron and not ascorbic acid There was no evidence that the cases were scorbutic and the diagnosis was made on low plasma ascorbic acid measurements Croft and Snorf [96] have been cited as presenting evidence that the anæmia of scurvy

may be due to lack of factors other than ascorbic acid. Actually there was no evidence that their patients suffered from scurvy; they suffered from a number of other diseases and had a low plasma ascorbic acid. There are no characteristic hæmatological findings in scurvy. The anæmia when it does occur conforms to no particular type; it may be macrocytic, normocytic or microcytic. Mild hypochromia is common but not constant. McMillan and Inglis [93], in a study of forty three scorbutic subjects, found macrocytic anæmia in two, normocytic anæmia in eighteen, microcytic anæmia in fourteen, and microcytic hypochromic anæmia in six. There are no characteristic changes in the bone marrow, which is normoblastic [84] and normally cellular or hypercellular [103]. Differential counts of nucleated marrow cells show a relative increase in normoblasts [103]. The bone marrow has also been described as hyperplastic [86], hypoplastic [95] and megaloblastic [98]. Vilter and his co workers [103] noted signs suggesting accelerated blood destruction in scorbutic patients, namely reticulocytosis, slight jaundice and

... These signs and the anæmia ... ascorbic acid ... part ... bile pigments [933] ... scurvy improves when the patient is put to bed and given a diet restricted in ascorbic acid [84, 103].

The conflicting reports on scorbutic anæmia that have ... may be explained. Scurvy, like other diseases of malnutrition, is probably a multiple deficiency state, and lack of other factors as well as ascorbic acid may influence blood formation. Infection and hæmorrhage may also play a part.

The possibility that ascorbic acid may influence the absorption and utilization of iron cannot be overlooked [109, 116]. ... [1631] has ... that anæmia in subjects with ascorbic acid deficiency did not respond to ascorbic acid alone, but did to ascorbic acid and iron. The administration of ascorbic acid increases the iron level in the blood [934].

It has been supposed that ascorbic acid aids the absorption of iron from the gut, from which it is absorbed in the ferrous state. Bergheim and Kirch [118] state that ascorbic acid reduces iron to the ferrous state in the stomach. Totterman [113] found no correlation between iron and ascorbic acid metabolism in ... no change in the s... nor had prolonge... iron in patients with infections, in which the latter is lowered. Totterman also found anæmia resulting from infection to be refractory to treatment with iron and ascorbic acid. These results are at variance with those of Albers [106] who states that ascorbic acid given intravenously to pregnant women causes a rise in the serum iron within three hours. Others have reported a rise in the serum iron following the administration of ascorbic acid [934]. In patients with anæmia due to iron deficiency injection of ascorbic acid caused a fall in the serum iron, after treatment ... the serum iron rose. The serum iron in patients ... tion after treatment with ascorbi... [115] the utilization of iron by patients with rheumatoid arthritis is not materially improved by the administration of ascorbic acid.

Ascorbic acid may be essential for the conversion of folic acid to folinic acid [117] and might therefore affect hæmatopoiesis indirectly. According to May and his co workers [131] lack of ascorbic acid plays a part in the ætiology of the megaloblastic anæmia of infancy, probably by interfering with the utilization of folic acid. Barron [101] suggests that ascorbic acid may play a role in m... level because it ... d experimentally ... ith ascorbic acid ... described on p. 480. It has no effect on polycythæmia due to continued exposure to lo...

The administration of doses of 100 to 300 mg of ascorbic acid produces changes in the blood chemistry—a decrease in blood sodium and chloride and an increase in blood potassium [316]

ASCORBIC ACID, INFECTION AND IMMUNITY

Blood Levels and Excretion in Infection In infections there is a lowering of the blood ascorbic acid and diminished excretion This has been demonstrated in animals and man in a number of infections such as tuberculosis pneumonia diphtheria and rheumatism [119 127] Thus Faulkner and Taylor [123] found that the average serum ascorbic acid of a group of healthy subjects was 1 31 mg per 100 ml whereas in patients with infectious diseases it was 0 65 mg per 100 ml The organs of animals suffering from infections contain considerably less ascorbic acid than those of healthy control animals [138] The lowered blood ascorbic acid and lowered excretion are not due to pyrexia *per se* but due to the infectious process [152]

This fall in the plasma ascorbic acid and diminished excretion in infections can be partly accounted for by the migration of the vitamin to the white blood cells (p 420) and the adrenals (p 430)

Ascorbic Acid and Immunity There is some evidence to show that ascorbic acid may be concerned with acquired immunity It is probably concerned with antibody formation Jusatz [149] found that the addition of 100 mg of ascorbic acid to an immunizing dose of horse protein caused a five to seven fold increase in the specific precipitin production in rabbits This was confirmed by Madison and Manwaring [153] and others [141 142 148 153] Cameron [140] found that the blood of guinea pigs treated with diphtheria toxoid and receiving 3 6 mg of ascorbic acid daily contained more antitoxin than controls similarly treated but receiving only 0 9 mg of the vitamin daily Birkhaug [150] claimed that supplements of 10 mg ascorbic acid produced a significant inhibition of the tuberculin reaction in guinea pigs He noted that the inhibition of the tuberculin reaction was correlated with the urinary excretion of ascorbic acid and the amount of the latter [illegible] and Steenken [151] were unable to confirm [illegible] others [818 819] found that the adminis [illegible] ous guinea pigs increases the tolerance to repeated large doses of tuberculin and Busing [154] observed that the administration of ascorbic acid to rats or rabbits infected with pneumococci or staphylococci increased the survival time

Much of the confusion on the subject arises from the failure to recognize that conclusions drawn from studies on one species (e g the guinea pig) that needs an exogenous supply of ascorbic acid cannot be applied to others (e g the rat) that synthesize their own ascorbic acid and cannot develop the symptoms of deficiency The latter species can be used to study the effects of an excess of ascorbic acid but never a deficiency Another source of confusion is failure to distinguish between the response following a single injection of an antigen (e g the *primary response* in the case of diphtheria toxoid) and the response after the animal has received one or more doses (secondary response) The blood antibody response to the first dose of antigen is small slow in developing rises and falls rapidly and is less affected by the amount of antigen than the secondary response The blood antibody response to more than one dose of antigen is large develops quickly rises rapidly is maintained for a relatively long time and its magnitude depends *on the dose of the antigen* Long [130] has shown that the primary antitoxin response of guinea pigs deficient in ascorbic acid to alum precipitated diphtheria toxoid is not significantly reduced whereas the secondary response is

The effect of ascorbic acid on the immune state is obscure The small effect of a deficiency *on the primary response* suggests that ascorbic acid is

concerned more with the metabolic systems controlling the production of antibodies in cells already in a state of secondary responsiveness than with the response of those cells to primary conditioning by the antigen (Long)

Ascorbic Acid and Bactericidal and Antitoxic Action Ascorbic acid has bacteriostatic and bactericidal activity In suitable concentration it prevents the development of pneumococci streptococci staphylococci *Hæmophilus pertussis Clostridium tet*

are destroyed These c

those present in hum

action The bactericidal action of blood is in fact independent of its ascorbic acid content [815] and if the vitamin is given to human subjects with low ascorbic acid levels it does not result in an increase in the bactericidal action against staphylococci *B coli* and *S typhosum* [160 169] If the ascorbic acid in blood is oxidized there is no decrease in its bactericidal action against the same organisms

Ascorbic acid inactivates certain toxins e g those of *Clostridium œdematiens Cl histolyticus Cl tetani B dysenteriæ* and *H pertussis* [115–160] These toxins are not only neutralized *in vitro* but if the ascorbic acid given is *in vivo* it can raise the resistance against toxins Certain strains of poliomyelitis virus are inactivated by ascorbic acid and according to Jungeblut [161] it has some curative effect *in vivo* in infected monkeys although this is disputed by Sabin [162] Ascorbic acid has a virucidal action on influenza A virus [136]

The toxin of *Corynebacterium diphtheriæ* particularly affects the adrenal glands in which the level of ascorbic acid falls (p 430) It has been suggested that ascorb c acid and the suprarenal cortex may play an important part in the anti infective processes of the body (p 430) Many workers have stated that the degree of virulence of a standardized diphtheria toxin as measured by guinea pig assay is dependent on the ascorbic acid intake of the animal and that an increased intake of the vitamin gives increased protection [164–166] According to Jungeblut [817] diphtheria toxin is inactivated *in vitro* by ascorbic acid particularly in the presence of cupric ions this was confirmed by Willison [936] who found that 2M L D of diphtheria toxin is detoxicated by 1 mg of ascorbic acid at 37° C

Zilva [170] Torrance [171] and others [207] have been unable to confirm these observations Zilva claims that guinea pigs treated with ascorbic acid show no more resistance to diphtheria than do deficient controls Torrance could not confirm the inactivation of diphtheria toxin by ascorbic acid nor

period of incubation and to the use of the wrong *p*H

Ascorbic Acid and Complement Between 1938 and 1940 several reports were published establishing a d rect correlation between the ascorbic acid level of the blood and its complement activity Complement which is a

components

and one of

that *in vivo*

there is a correlation at concentrations of ascorbic acid below 1 mg per 100 ml of serum They claim that in scurvy the administration of graded doses of ascorbic acid is paralleled by a corresponding increase in the complement titre Simola and Brunius [516] and Marsh [173] also claimed to have observed a lowering of complement titre in animals with ascorbic acid deficiency Chu and Chow [174] recorded similar observations on thirty eight patients on basal diets receiving increasing quantities of ascorbic acid

More recent work has failed to confirm these observations which were based on average results without statistical evaluation Zilva [176] and Chakraborty [469] could find no significant change in the complement titre

of scorbutic guinea pigs Spink Agnew and Michelson [855] showed that a fall in plasma ascorbic acid in both guinea pigs and adult humans is not accompanied by a reduction in the complement titre and that neither the *in vitro* nor *in vivo* addition of ascorbic acid results in change of complement titre They further state that ascorbic acid can be removed chemically from blood without changing the titre of complement Similar observations were made by Rice and Boulanger [131] Kodicek and Traub [937] were unable to find any significant change in complement in guinea pigs partially or completely deficient in ascorbic acid These observations are in agreement with [illegible] [68] [illegible]

[illegible]

were estimated but they were unable to establish any linear relationship between the two in health or disease when the two factors were plotted there was a random distribution of the points Further indirect evidence of lack of any correlation between ascorbic acid and complement titre is the observation that although newborn infants have a higher plasma ascorbic acid concentration than their mothers (p 444) they have a significantly lower complement activity [937]

Feller and his associates [856] have also studied the following immunological phenomena in relation to ascorbic acid and also vitamin A nutrition

(*a*) Capacity of nasal secretion to inactivate influenza virus
(*b*) Titre in blood serum of neutralizing antibodies for influenza virus
(*c*) Activity of lysozyme in the nasal secretion
(*d*) Phagocytic activity
(*e*) Complement titre of blood serum and of polymorphonuclear neutrophile leucocytes in whole blood for pneumococci

The results of the various immunological tests were not significantly influenced by marked changes in the plasma levels of ascorbic acid or vitamin A or by a period of severe ascorbic acid deficiency followed by a large excess of the vitamin The authors of the work point out however that because of the multiplicity of factors involved in the mechanisms of virulence susceptibility and resistance broad conclusions cannot be drawn from the study

Ascorbic Acid and Leucocytosis Ascorbic acid is taken up in large quantities by the leucocytes of the blood [182] and in concentrations of 0 25 to 1 p p m stimulates their respiration [268] It has been demonstrated by special cytological methods by Tonutti and Matzner [102] in the leucocytes of the lungs of guinea pigs with pneumonia They observed an increase in the alveolar phagocytes which were laden with ascorbic acid granules which sometimes contained as much as 0 3 per cent The alveolar exudate also contained considerable ascorbic acid in its cells According to Cuttle [104] considerable quantities of ascorbic acid are stored in the white blood cells since in leucocytosis an excessive amount of the vitamin can be absorbed and retained and the amount in the blood cells is increased These abnormalities bear a direct relationship to the number of circulating leucocytes The increased utilization of ascorbic acid in infections (p 418) may be due to the accompanying leucocytosis When erythrocytes and leucocytes compete for ascorbic acid *in vitro* it is taken up preferentially by the leucocytes [843]

Cottingham and Mills [939] observed that ascorbic acid deficiency severe enough to retard growth produces a corresponding reduction in phagocytic activity The leucocytes of adequately fed guinea pigs took up an average of 18 3 micro organisms per cell *in vitro* with ninety nine per cent of the cells showing evidence of bacterial destruction by the end of one hour On a diet deficient in ascorbic acid phagocytosis was reduced to 7 3 bacteria per cell with intracellular digestion reduced to seventy four per cent Nungester and Ames [132] observed that in guinea pigs deficient in ascorbic acid the peritoneal exudate was tinged with blood and contained few white

cells They found that the phagocytic activity of these cells was related to their ascorbic acid content This correlates with the observation of Perla and Marmorston [143] that the cellular response to intraperitoneal irritation is poor in scorbutic guinea pigs

It has been stated that the injection of ascorbic acid provokes a leucocytosis in infective states or in leucopenia [105] From histological studies on experimental fractures in rabbits it would appear that the injection of ascorbic acid causes an increased proliferation of the reticulo endothelial elements [137] This occurs even in animals on a diet containing an adequate supply of the vitamin Meyer [181] determined the opsonic index of three subjects and found that the injection of ascorbic acid caused a marked rise in the phagocyte count in one case as much as six times the normal The injection of doses of from 50 to 750 mg of ascorbic acid into normal individuals is stated to cause considerable leucocytosis e g up to an increase of sixty eight per cent [854]

Crandon [68] found that whilst on a scorbutic diet his white cell count fell from 5 000 to 3 500 After an injection of 1 000 mg of ascorbic acid it rose to 5 000 and later to 9 000 Faulkner [184] observed that the administration of large doses of ascorbic acid in various infective conditions was accompanied by appreciable reticulocyte responses analogous to those following the administration of ascorbic acid to patients with the anæmia of scurvy (p 453)

Bacchus and Toompas [107] observed that ascorbic acid did not produce a leucocytosis in the rat which synthesizes its own ascorbic acid although it did produce a considerable eosinophilia if administered with adrenaline

This work on ascorbic acid and infection is difficult to interpret The bulk of the evidence shows that it plays some part in immunity phenomena and what is vaguely termed resistance to infection

Ascorbic Acid as a Detoxicating Agent *Arsphenamines* It was observed by Sulzberger and Oser [185] in 1935 that large doses of ascorbic acid reduced and inhibited the susceptibility of the skin of the guinea pig to experimental sensitization with neoarsphenamine This was confirmed by Cormia [186] who states that possibly sensitization does occur but that ascorbic acid inhibits the cutaneous reactions McDonald and Johnson [270] state that ascorbic acid has some protective action against arsphenamine reactions in guinea pigs but they could find no relation between the amount administered and the degree of protection These results were questioned by Cohen [187] who in carefully controlled studies showed that there was no difference in sensitivity to neoarsphenamine between guinea pigs on a diet deficient in ascorbic acid and those receiving an adequate supply Chapman and Morrell [192] obtained results exactly opposed to those previously described their guinea pigs on a diet low in ascorbic acid were actually less sensitive to arsphenamine than those on a normal diet

T

ascorbic acid are injected in the same solution intravenously, and that if the ascorbic acid is given two hours before the neoarsphenamine its protective action is lost They state that a dose of neoarsphenamine which represents LD_{50} kills only ten per cent of the animals if this is injected with an equal weight of sodium ascorbate The amount of ascorbic acid needed to exert a protective effect is between a quarter and an eighth of the weight of the arsphenamine or half a mole of ascorbic acid for a mole of neoarsphenamine These investigators state that at a level of three moles of ascorbic acid per one mole of neoarsphenamine the dose of the latter may be increased to 700 mg per kilo with no greater toxicity than that produced in controls

of scorbutic guinea pigs Spink, Agnew and Michelson [855] showed that a fall in plasma ascorbic acid in both guinea pigs and adult humans is not accompanied by a reduction in the complement titre, and that neither the *in vitro* nor *in vivo* addition of ascorbic acid results in change of complement titre They further state that ascorbic acid can be removed chemically from blood without changing the titre of complement Similar observations were made by *Rice and Boulanger* [131] *Kodicek and Traub* [937] were unable to find any significant change in complement in guinea pigs partially or completely deficient in ascorbic acid These observations are in agreement with [illegible]

[illegible]

were estimated but they were unable to establish any linear relationship between the two in health or disease, when the two factors were plotted there was a random distribution of the points Further indirect evidence of lack of any correlation between ascorbic acid and complement titre is the observation that although newborn infants have a higher plasma ascorbic acid concentration than their mothers (p 444) they have a significantly lower complement activity [937]

Feller and his associates [856] have also studied the following immunological phenomena in relation to ascorbic acid and also vitamin A nutrition

(*a*) Capacity of nasal secretion to inactivate influenza virus

(*b*) Titre in blood serum of neutralizing antibodies for influenza virus

(*c*) Activity of lysozyme in the nasal secretion

(*d*) Phagocytic activity

(*e*) Complement titre of blood serum and of polymorphonuclear neutrophile leucocytes in whole blood for pneumococci

The results of the various immunological tests were not significantly influenced by marked changes in the plasma levels of ascorbic acid or vitamin A or by a period of severe ascorbic acid deficiency followed by a large excess of the vitamin The authors of the work point out, however, that because of the multiplicity of factors involved in the mechanisms of virulence susceptibility and resistance broad conclusions cannot be drawn from the study

Ascorbic Acid and Leucocytosis Ascorbic acid is taken up in large quantities by the leucocytes of the blood [182] and in concentrations of 0 25 to 1 p p m stimulates their respiration [268] It has been demonstrated by special cytological methods by Tonutti and Matzner [102] in the leucocytes of the lungs of guinea pigs with pneumonia They observed an increase in the alveolar phagocytes which were laden with ascorbic acid granules which sometimes contained as much as 0 3 per cent The alveolar exudate also contained considerable ascorbic acid in its cells According to Cuttle [104] considerable quantities of ascorbic acid are stored in the white blood cells since in leucocytosis an excessive amount of the vitamin can be absorbed and retained and the amount in the blood cells is increased These abnormalities bear a direct relationship to the number of circulating leucocytes The increased utilization of ascorbic acid in infections (p 418) may be due to the accompanying leucocytosis When erythrocytes and leucocytes compete for ascorbic acid *in vitro* it is taken up preferentially by the leucocytes [843]

Cottingham and Mills [939] observed that ascorbic acid deficiency severe enough to retard growth produces a corresponding reduction in phagocytic activity The leucocytes of adequately fed guinea pigs took up an average of 18 3 micro organisms per cell *in vitro*, with ninety nine per cent of the cells showing evidence of bacterial destruction by the end of one hour On a diet deficient in ascorbic acid phagocytosis was reduced to 7 3 bacteria per cell, with intracellular digestion reduced to seventy four per cent Nungester and Ames [132] observed that in guinea pigs deficient in ascorbic acid the peritoneal exudate was tinged with blood and contained few white

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It has been stated that the injection of ascorbic acid provokes a leuco cytosis in infective states or in leucopenia [105] From histological studies on experimental fractures in rabbits it would appear that the injection of ascorbic acid causes an increased proliferation of the reticulo endothelial elements [137] This occurs even in animals on a diet containing an adequate supply of the vitamin Meyer [181] determined the opsonic index of three subjects and found that the injection of ascorbic acid caused a marked rise in the phagocyte count in one case as much as six times the normal The injection of doses of from 50 to 750 mg of ascorbic acid into normal individuals is stated to cause considerable leucocytosis e g up to an increase of sixty eight per cent [854]

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receiving 400 mg. per kilo If the ascorbic acid is injected simultaneously at another site the detoxifying action is somewhat decreased but not entirely eliminated. Friend and Ivy [133] found that ascorbic acid had a protective effect against the organic arsenical chlorarsen if ascorbic acid were given for nine days before and for three days after the arsenical

Evidence for the increased tolerance of the human organism to the

toxic reactions due to parenteral arsenic therapy with oral or intravenous doses of 100 to 300 mg of ascorbic acid daily, and he states that the period of treatment is considerably reduced. Intravenous or intramuscular doses of 300 mg a day controlled many of the symptoms of neoarsphenamine intolerance in twenty-two cases quoted by Welker [250]. Large intravenous doses of ascorbic acid (500 mg a day) followed by high maintenance doses of 100 to 200 mg by mouth were given by Cormia [193] to patients who had previously suffered from neoarsphenamine dermatitis He found that they were able to tolerate more of the same arsenical without further reactions, although he admits that his results were not so spectacular as those of Dainow Actual cases of neoarsphenamine dermatitis cleared up without fourteen to eighteen days after giving 100 to 200 mg. of ascorbic acid by mouth

Falconer, Epstein, and Mills [194] studied a group of seven patients in whom attacks of thrombopenic purpura repeatedly occurred after the administration of neoarsphenamine and bismarsen At no time and in none of the patients was any appreciable modification of sensitivity to the drugs observed during or after the administration of 100 mg doses of ascorbic acid

The blood ascorbic acid of a number of patients showing signs of sensitiveness to neoarsphenamine was examined by Friend and Marquis [195] The values were from 0 13 to 0 35 mg —well below normal levels It was concluded that a lov ... result of a toxic reaction to the neoarsphenamine ... actor to such reactions This view is open to ... blood values were not determined, and four out of twelve patients receiving arsphenamine without reactions had low ascorbic acid blood levels Farmer and his colleagues [196] have also demonstrated that patients hypersensitive to neoarsphenamine show very low ascorbic acid blood levels In patients showing severe symptoms of intolerance a fall in the blood ascorbic acid occurred in spite of the oral administration of the vitamin during treatment It was frequently

Weber [820, 821] also state that patients showing sensitivity to ... have low ascorbic acid levels. By giving 300 mg. of this vitamin a day orally and 300 to 500 mg intravenously every other day they claim that the sens...ty of such patients is diminished

on

fou

and mapharsen A number of patients were patch tested with ... amine, and positive reactors retested with the drug to which ascorbic acid had been added Not a trace of reaction was found in thirty two out of thirty eight who formerly reacted to neoarsphenamine alone Further studies ... and his co-workers suggest that the majority of hypersensitive

sphenamine This work

Beerman and his co workers [197] claim th... ...inically the incidence of

reactions to antisyphilitic arsenicals is reduced fifty eight per cent if the substances are dissolved in a one per cent solution of methyl glucamine ascorbate before administration White [216] has been unable to confirm this in animal tests

Lead and Gold The plasma ascorbic acid of the rat is markedly decreased by the intraperitoneal administration of gold chloride [134] Sande [198] observed that the ascorbic acid content of the organs of guinea pigs that had received injections of gold salts was much lower than that of control animals Three patients after treatment with gold developed symptoms of intolerance and a lowered capillary resistance, one patient also had signs of liver injury After treatment with 100 to 200 mg of ascorbic acid a day given intravenously the symptoms of intolerance rapidly disappeared Dainow [188] describes the treatment of erythrodermia due to gold
0 2 gram of ascorbic acid Cohen and
to observe any beneficial effect from
five patients receiving 118 courses of

Studies on lead poisoning were made by Holmes and his co workers [199] who observed four hundred men exposed to lead over a period of a year Thirty four had symptoms of lead poisoning Half were treated with additional doses of 100 to 200 r eks and in most the vitamin was the symptoms of lead poisoning tion nervousness) and restoring the blood picture than in the controls who received the usual therapy with calcium Marchmont Robinson [245] from a study of over three hundred lead workers concluded that 50 mg of ascorbic acid a day protected them against the effects of chronic lead absorption Pillemer [201] in a well controlled experiment with guinea pigs found that in two series of fo intake of ascorbic acid the degree nth s ingestion of lead carbonate two groups of twenty four animals saturated with the vitamin

Dannenberg [200] Evans [943] and their co workers were unable to confirm these observations Evans and his colleagues studied a group of four hundred workers in a tetraethyl lead factory The level of ascorbic acid nutrition was low The administration of 100 mg daily failed to have any effect on the lead concentration in the blood or on its elimination in the feces and urine No difference was noted in the physical condition number and severity of complaints erythrocyte count number of stippled erythrocytes or

effects of certain drugs can be prevented or minimized by the administration of ascorbic acid Thus Dainow [202] and Bickel [203] state that some of the toxic effects of the sulphonamides (particularly sulphapyridine) can be prevented or at any rate relieved by the simultaneous administration of ascorbic acid in daily doses of 0 5 gram given intravenously According to Dainow the urinary excretion of ascorbic acid is considerably decreased in patients receiving sulphonamide and in animals there is a fall in the ascorbic acid content of the brain liver and testicles He argues that the normal ascorbic acid reserves are mobilized to detoxicate the sulphonamides Dunlop [204] has been unable to confirm Dainow s statement that ascorbic acid increases the tolerance of patients to sulphapyridine and others have found no difference in the survival rate of rats and guinea pigs treated with toxic doses of sulphanilamide alone and with sulphanilamide and ascorbic acid [825 1058] Pelner [1014] gave full doses of sulphadiazine to fifty

patients together with 100 mg of ascorbic acid daily, and stated that none of them suffered from any reactions As no controls were observed it cannot be said that ascorbic acid definitely exerted any protective action Contrary to Damow's observations, Holmes [932] Kinnunen [168], Ekman [163] and Longenecker [167] observed an increased urinary excretion of ascorbic acid in patients given sulphathiazole, and recommended supplements of 100 mg daily to allow for this According to Ekman increased excretion is due to increased synthesis of the vitamin in the tissues He states that ascorbic acid is concerned with the oxidation and detoxification of cyclic compounds in the body

It has also been stated that ascorbic acid diminishes the toxicity of such substances as anaesthetics [213] benzene [177, 205, 206] trichlorethylene, T N T [823] barium [178] and barbiturates [949]

There is some doubt about the detoxifying effect of ascorbic acid on T N T

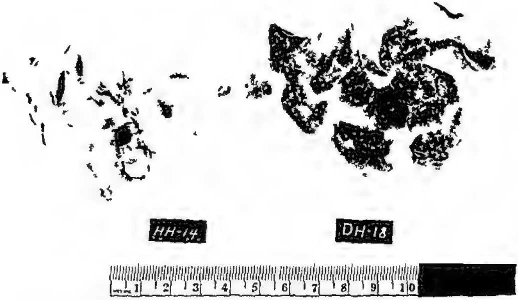


FIG 148 The protective action of ascorbic acid against hepatotoxic agents

daily injections
is shown on the r
was practically
however was p
It was sixty eig

Smith and his colleagues [950] were unable to observe any protective effect in cats rats or guinea pigs In the United States a recommendation has been made that munition workers exposed to T N T should receive at least 100 mg of ascorbic acid daily

Ascorbic acid appears to increase the rate of excretion of amphetamine (benzedrine) [252] and to increase the rate of metabolism

Kimball [210] and Frankel [211] believe that the toxic effects of sodium diphenylhydantoin (soluble phenytoin) a drug used in the treatment of epilepsy, are more pronounced in patients deficient in ascorbic acid According to Drake [599] in the experimental animal sodium diphenyl hydantoin causes an increased excretion of ascorbic acid in the urine, and a lowering of the body reserves of the vitamin [599] Gruhzit [212], however, ... effect whatsoever ... ive reported that it has no effect on the ascorbic acid blood le... s [682] An

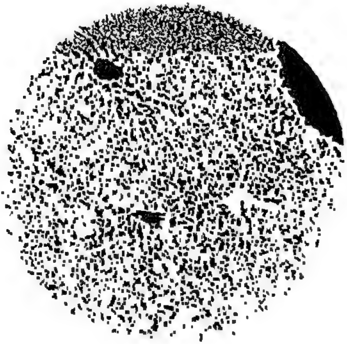


FIG 149 The protective action of ascorbic acid against hepatotoxic agents *A section of the liver of guinea pig HH-14 (see Fig 148), given 30 mg. of* ascorbic acid daily and then 25 mg. of hydrazine for two days Hematoxylin and eosin stain (× 100) The cellular appearance is almost normal. Under a magnification of × 400 some hydropic changes in the cytoplasm are visible

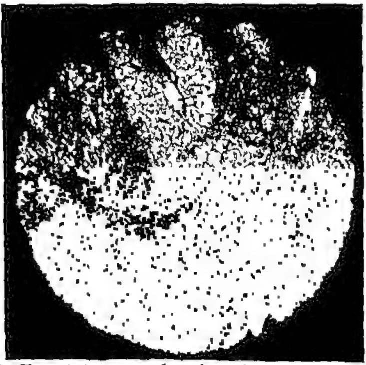


FIG. 150. The protective action of ascorbic acid against hepatotoxic agents.
[illegible]

necrosis.

examination of epileptics receiving sodium diphenylhydantoin revealed that the plasma ascorbic acid was not influenced in any way by the type of therapy received nor did the long continued administration of sodium diphenyl hydantoin have any effect on the plasma ascorbic acid Gruhzit believes that the low levels of vitamin found in the plasma of epileptics receiving the drug is not due to the latter but to an inadequate intake of ascorbic acid Ziskin [824] observed that gum hyperplasia resulting from the administration of diphenylhydantoin is not significantly altered by giving ascorbic acid

Other workers have failed to confirm the earlier observations of Kimball and Frankel [947, 948] Emmett and co workers [948] have conducted a careful investigation on the effect of phenytoin on the weight gross patho logical changes, plasma ascorbic acid levels and ascorbic acid content of the tissues of guinea pigs and they concluded that the drug had no significant effect on the utilization of ascorbic acid in this animal

Beyer [952] has shown that ascorbic acid protects against hepatotoxic agents such as hydrazine (Figs 148 to 150) Guinea pigs deficient in ascorbic acid showed an average of fifty per cent more fat in their livers than guinea pigs receiving adequate ascorbic acid when both groups were fed hydrazine Gross and microscopic evidence confirmed the severity of fatty degeneration in the animals deficient in ascorbic acid

Chapman and Shaffer [179] state that the administration of ascorbic acid prior to or simultaneously with some mercurial diuretics e g mercuhydrin reduces their toxicity Vauthey [214] noted an increased survival rate in guinea pigs poisoned with mercury given ascorbic acid Frommel and his co workers [208] however failed to observe any protective action when guinea pigs poisoned with mercury were given ascorbic acid Ekman [180] has shown that the toxic effects following the exposure of guinea pigs to benzene vapour can be diminished by ascorbic acid Large doses are less effective than moderate doses Ekman considers that the benzene is detoxified by hydrogen peroxide formed by oxidation of ascorbic acid

The toxicity of procaine is increased in guinea pigs deficient in ascorbic acid [183], and the prolongation of sleeping time in guinea pigs depleted of ascorbic acid after the administration of certain barbiturates is reversed by giving ascorbic acid [189] According to Frommel and his co workers [190] analgesics and antipyretics produce a fall in the ascorbic acid content of the

flavoprotein level and as ascorbic acid has a redox potential which is higher than that of the latter it might provide an alternate pathway for the transport of hydrogen when normal metabolism is blocked

H_2
Substrate ——→ co enzymes ——→ flavoprotein —— Ascorbic acid
↓
cytochrome b
Possible site of action of thiopentone
↓
H_2O →2H+O→cytochrome oxidase→cytochrome A→cytochrome C ← (Ascorbic acid)

The effect of ascorbic acid is augmented by cytochrome C

Role of Ascorbic Acid in Cellular Oxidations It has been considered that ascorbic acid is a component of a reversible oxidation reduction system in the body, acting as a hydrogen transporter The formation of hydrogen peroxide has been demonstrated during the autoxidation of ascorbic acid in the presence of water [286]

O ——> ascorbic acid oxidase <—— ascorbic acid <—— 2H

H_2O_2

H_2O + oxidized substrate

The cytochrome indophenol oxidase system has been shown to act as a catalyst in the aerobic oxidation of ascorbic acid [219] It is suggested that

Prunty and Vass [274]
human red blood cells

Harrison [254] von Euler and Klussman [255] state that slices of fresh tissues from scorbutic animals have a lower oxygen uptake than controls and that it is restored by adding ascorbic acid This could not be confirmed by Stotz and his co workers [220] The oxidation of certain fatty acids in the liver proceeds at a higher rate in the presence of ascorbic acid [224]

Ascorbic Acid and Amino Acid Metabolism

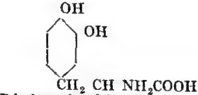
As far back as 1930 Szent Gyorgyi [231] wrote · "Hexuronic acid (i e ascorbic acid) completely inhibits the formation of pigment in all systems in which a melanoid pigment is formed through the oxidation of a phenol" Abderhalden [232] showed that *in vitro* ascorbic acid inhibited pigment formation from adrenaline and from 3 4 dihydroxyphenylalanine ("dopa") both in the presence and absence of the enzyme tyrosinase This has been confirmed by others Many investigators have reported a decrease in the deposition of pigment in Addison's disease and a bleaching of existing pigmentation after administering large quantities of ascorbic acid (p 430) This bleaching effect has been attributed to direct reduction of melanin by ascorbic acid [872]

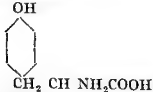
These observations suggested ascorbic acid might play a rôle in amino

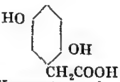
of synthesizing melanin, were used no deficiency symptoms were produced This suggests that ascorbic acid is used in the transformation of tyrosine and dopa into melanin

Alkaptonuria (phenylketonuria tyrosinosis) is normally encountered as an inborn error of metabolism There is a defect in the enzyme systems involved in the oxidation of phenylalanine or tyrosine and their derivatives, such as homogentisic acid, which appears in the urine and causes it to darken considerably on standing Alkaptonuria has been produced experimentally in the guinea pig by


Phenylalanine

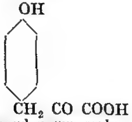

Dihydroxyphenylalanine


Tyrosine

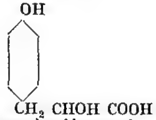

Homogentisic acid

Sealock and his co workers [217] by administering large amounts of phenyl alanine, tyrosine or dopa in the absence of adequate amounts of ascorbic acid It has also been produced in the white rat Homogentisic acid is therefore a step in the normal pathway of phenylalanine and tyrosine metabolism The amount present in the urine is inversely proportional to the ascorbic acid intake, and when this is adequate homogentisic acid disappears from the urine [225] This occurs in man Ascorbic acid is however, ineffective in correcting the metabolic defect when given to subjects suffering from hereditary alkaptonuria [218]

Premature infants fed on cow's milk are unable to metabolize the tyrosine and phenylalanine in the milk proteins in the absence of ascorbic acid They excrete *p* hydroxy phenylpyruvic and *p* hydroxyphenyl lactic acids [272]



p Hydroxyphenyl pyruvic acid



p Hydroxyphenyl lactic acid

Full term infants show this error of metabolism only if they are given additional tyrosine or phenylalanine In both full term and premature infants it is corrected by ascorbic acid Pteroylglutamic acid also prevents it [238] An enzyme system in liver oxidizes tyrosine, but this is absent from the livers of scorbutic animals The scorbutic liver however regains its power to oxidize tyrosine *in vitro* and *in vivo* after the addition of ascorbic acid [226] The oxidation of dopa" occurs chiefly in the kidney rather than the liver [221, 223] According to Painter and Zilva [230] the normal metabolism of tyrosine is mediated by intestinal bacteria the accumulation of hydroxyphenyl compounds being due to the absence of the modifying influence of ascorbic acid on the intestinal flora responsible for the breakdown of tyrosines The oxidation of tyrosine to 'dopa" *in vitro* by ascorbic acid and oxygen has been demonstrated [229]

Deranged tyrosine metabolism is also seen in human scurvy [238, 251] Scorbutic infants and adults excrete in the urine *p* hydroxyphenyl compounds (*p* hydroxypyruvic acid and *p* hydroxyphenyl lactic acid) when given tyrosine, these disappear when ascorbic acid and pteroylglutamic acid are administered [236 238] Increased excretion of *p* hydroxyphenyl compounds in scorbutic monkeys is diminished by ascorbic acid but not by pteroyl glutamic acid

Benzoquinoneacetic acid has been identified by Fishberg [257] in the urine of subjects on diets deficient in ascorbic acid It is an intermediate in the catabolism of tyrosine and phenylalanine

If a high protein diet is fed to rats the ascorbic acid in the blood and in those tissues that metabolize large amounts of amino acids (liver kidney muscle) is lower than when diets high in fat or carbohydrate are eaten [279] Administration of ascorbic acid increases the concentration in the active organs but not in the blood These observations suggest that tissues metabolizing large amounts of amino acids have increased utilization of ascorbic acid

[illegible] The cytochemical [illegible]

in the body contains as much ascorbic acid as the [illegible] per gram of fresh tissue) Large quantities are present in the *Golgi* apparatus

Demonstration of Ascorbic Acid in Tissues by Silver Staining Technique

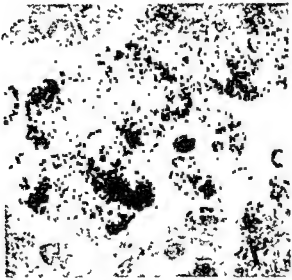


Fig 151. Ascorbic Acid in the Pituitary Gland. Deposits of silver representing granules of ascorbic acid in chromophil cells

Fig. 152 Ascorbic Acid in Corpus Luteum of Dog. After staining for fifteen minutes with 0 4 per cent. silver nitrate solution.

Fig 153 Ascorbic Acid in the Pituitary of a Dog. After staining for fifteen minutes with 0 4 per cent silver nitrate solution

Fig. 154. Ascorbic Acid in Interstitial Cells of Testis After staining for fifteen minutes with 0 4 per cent. silver nitrate solution.

of the adrenal cortex [271] The connection between ascorbic acid adrenaline and pigment formation has already been mentioned (p 429) The administration of ascorbic acid to guinea pigs is said to increase the adrenaline content of the adrenals [244] Vogt [282], however, has found no correlation between the amount of adrenaline secreted and the ascorbic acid content of the blood leaving the adrenal gland Many workers claim that low blood and urinary ascorbic acid values occur in patients with Addison's disease [237, 239], and they have observed a striking diminution in the pigmentation of the skin that occurs in this disease after the administration of ascorbic acid (500 *mg* daily) [235, 237, 239, 872] The evidence on the storage and excretion of ascorbic acid in patients with Addison's disease is conflicting Sendroy and Miller [240] state that the urinary excretion of ascorbic acid is low because of renal insufficiency Jenovese and others [241] state that although the urinary excretion of ascorbic acid is low, blood plasma levels are within normal limits

From cytochemical studies Deane and Morse [291] and Sayers [862 863] have demonstrated a close connection between the adrenocorticotropic hormone of the pituitary (A C T H) and ascorbic acid A single dose of A C T H given to a rat or guinea pig causes a fall in the cholesterol and ascorbic acid of the adrenals, it has been suggested that both of these are essential for the synthesis and release of adrenal cortical hormones [320] It appears that there is an optimal ratio of adrenal cholesterol to adrenal ascorbic acid for normal steroid production in the adrenal cortex [1018] The fall in the ascorbic acid content of the adrenals of the hypophysectomized rat has been used by Sayers [296] for the assay of A C T H activity Various noxious stimuli, e g hæmorrhage, burns, cold, muscle trauma and nerve stimulation, and the administration of adrenaline [288] rapidly reduce the ascorbic acid in the adrenals [281] The subjection of rats to heat stress causes a fall in the adrenal ascorbic acid [477] This fall can be prevented by the previous administration of cortical hormone [283] The administration of salicylates also causes a fall in the ascorbic acid of the adrenals [1021], the

on the metabolism of ascorbic acid in man are contradictory, some observers reporting no significant changes in the urinary excretion *and others an in*creased excretion

Stefanini and Rosenthal [298] observed a fall in the plasma ascorbic acid and a low urinary excretion of the vitamin in two patients treated with A C T H, in both a hæmorrhagic diathesis occurred, suggestive of that seen in scurvy

Hyman and his co workers [299] studied the effect of A C T H and cortisone in ... Although A C T H caused a fall in the adrenal ascorbic ... ed the onset of scorbutic symptoms The wo ... suggests that there is an increase of adrenal cortical activity in scurvy as judged by adrenal hypertrophy and an increased excretion of ... from the increase of 17 ketosteroid excretion Both of these ... ortisone although this has no effect on the dis ... and Prunty, the 17 ketosteroid excretion is increased in the *early stages of scurvy*, and in the fully developed condition it is maximal, although this is denied by Banerjee and Deb [1025] Adrenal cortical activity in scurvy is increased by A C T H even in the absence of ascorbic acid

Schaffenburg Masson and Corcoran [56] found that cortisone also inhibits *many* of the manifestations of scurvy, probably because of its action on mesenchymal tissues ... C T H ... orn's to five patients with cl ... inis test) was the same as

Demonstration of Ascorbic Acid in Tissues by Silver Staining Technique

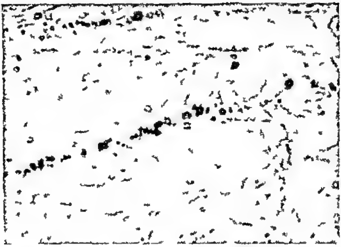


Fig 155 Ascorbic acid in Embryonic Nerve Tissue Ascorbic acid granules are seen scattered along a developing axone in a chicken embryo Silver stain (× 800)

Fig 156 Ascorbic acid in the Adrenal Cortex Cells from the secretory zone of the adrenal cortex of the rat showing ascorbic acid aggregated in the Golgi region of the cells Silver stain (× 2 000)

Fig 157 Ascorbic acid in the Adrenal Medulla Cell in the adrenal medulla of a cat showing ascorbic acid aggregated in the nucleus near the Golgi region Silver stain (× 1 200)

tering ascorbic acid Eisenstein and Shank [377] however observed a fall in the circulating eosinophils in scorbutic animals after administering A C T H Other tests by Treager showed a normal adrenal cortical activity (normal serum sodium and potassium) These observations suggest that ascorbic acid in the adrenal is not essential for the formation of the corticosteroids although theoretically the small amount of ascorbic acid still present [illegible] times present a syndrome crisis Perhaps it is only exhausted

Ascorbic acid given to animals exposed to stress exerts certain protective actions which are related to adrenal cortical function Thus the exposure of rats to cold normally causes an increase in weight of the adrenals which is prevented by giving ascorbic acid [304] the previous administration of ascorbic acid prevents signs of the alarm reaction in animals under the stress of adrenaline [107] the injection of ascorbic acid alters the eosinopenia of stress in intact rats and delays the eosinophilia characteristic of stress in adrenalectomized rats [308] under stress the excretion of ascorbic acid is greater than the intake but if A C T H is injected the urinary excretion of ascorbic acid falls and the plasma and cell levels rise [310] In normal dogs and rats the administration of A C T H cortisone or D O C A decreases the urinary excretion of ascorbic acid [316]

In the alarm reaction of the general Adaptation Syndrome (Selye) the ascorbic acid is aggregated peripherally in the cells of the adrenal cortex The resistance" phase is characterized by two types of cell in the cortex one with the ascorbic acid arranged peripherally in the cell the other with the ascorbic acid diffusely distributed in the cytoplasm In the exhaustion phase the cells of the cortex are enlarged but their ascorbic acid content is diminished [311]

When ascorbic acid is given to normal subjects in doses of 100 to 300 mg intravenously there is a fall in blood sodium and cholesterol and a rise in blood cholesterol and potassium (cf electrolytes in Addison s disease) if A C T H is administered at the same time it can prevent this disturbance in the blood electrolytes and facilitates storage [illegible] gland [316] Sayers [320] has expressed the [illegible] cholesterol take part in the formation of A C T [illegible] to understand as the administration of A C T H to the rat or guinea pig causes a fall in the adrenal cholesterol

The slow increase in the cell ascorbic acid compared with the plasma ascorbic acid suggests that something of a barrier exists between the cell and the plasma to the entry of ascorbic acid In the presence of A C T H more ascorbic acid passes into the cell and at a faster rate [323] It is of interest that ascorbic acid is conserved in the blood cell and not in the plasma in ascorbic acid deficiency (cf Crandon p 454) The plasma level can be lowered to zero but the cells still hold appreciable amounts The reduction of cell ascorbic acid in ascorbic acid deficient animals becomes even more difficult when A C T H is administered [316]

Pituitary A progressive fall in the plasma ascorbic acid similar to that seen after adrenalectomy occurs in the hypophysectomized rat [293] Stress reproduces the same effect After the administration of adrenal cortex extract the level reverts to normal According to Skelton and Fortier [293] the pituitary adrenal system plays a role in the increased synthesis of ascorbic acid facilitated by stress

Thyroid Most workers agree that the administration of thyroid thyroxine or the thyrotropic hormone results in a reduction in the ascorbic acid content of the liver adrenal thymus and kidney [258 261 265] Paal and Brecht [262] however observed a rise in the adrenal ascorbic acid A fall in tissue and plasma ascorbic acid after administering thyroid has also been reported [244] and what is surprising anti thyroid drugs such as

thiourea and thiouracil have the same effect [244] That ascorbic acid promotes the storage of glycogen is suggested by the work of Morelli and d'Ambrosio [289] and of Fischbach and Terbruggen [295] These workers could not observe any effect on the part of ascorbic acid on the glycogen of the livers of animals treated with thyroxine According to Hirsch [326] and Steffen and Zais [331] large doses of ascorbic acid partially prevent the fall in liver glycogen produced in guinea pigs or rats by thyroxine This conflicting evidence has since been re examined by Crabtree and Trikojus [333], who state that the effect of the administration of thyrotropic hormone or of thyroxine is uninfluenced by large doses of ascorbic acid, as judged by liver glycogen values, thyroid, adrenal and body weight and by histological examination of the thyroid They found that the liver can concentrate ascorbic acid when large doses of this are given at the same time as thyroid or thyrotropic hormone

Demole and Ippen [258] and Belasco and Murlin [243] stated that in the guinea pig the loss in weight caused by thyroxine can be checked by ascorbic acid provided enough is given In thyrotoxic patients the ascorbic acid excretion is said to be low [264] , the administration of large doses of ascorbic acid is without effect on the basal metabolic rate [259]

The effect of thyroxine on the ascorbic acid level in the adrenal is variable [260] First the level falls and then it rises The administration of anti thyroid drugs such as thiouracil results in an initial increase in the ascorbic acid of the adrenals, which atrophy [334] Thyroidectomy on the other hand produces a final decrease in the ascorbic acid concentration in the gland

Ascorbic Acid and Carbohydrate Metabolism The metabolism of carbo

increased it has been shown by removal of the adrenal medulla that adrenaline is not responsible for the lowered glucose tolerance [242] Banerjee and Deb [275] have suggested that the altered carbohydrate metabolism in scorbutic guinea pigs may be due to the combined deficiency of adrenal cortical hormone and insulin A diabetic type of glucose tolerance curve has been described in human subjects on low ascorbic acid intakes , this curve is said to return to normal on giving adequate ascorbic acid [665, 668]

Conflicting results have been reported on the effects of injecting ascorbic acid into normal human subjects A fall in blood sugar has been described after the intravenous injection of the vitamin [248, 669] although this has been denied by others [670] There is no evidence that the administration of ascorbic acid has any effect on the glucose tolerance of diabetics, even when given in large doses [257] The state of ascorbic acid nutrition in diabetics is no worse than that of normal controls [256] The injection of insulin into normal or diabetic subjects produces a fall in the plasma ascorbic acid and in the urinary excretion [249, 253] , this effect wears off and is followed by a rise in plasma concentration and increased excretion of ascorbic acid, and finally by a return to normal levels [247] There is no loss of ascorbic acid but a redistribution in the body. There is a transfer of ascorbic acid from the plasma to the white cell platelet layer of the blood

Dehydroascorbi T

rats produced by d

[1029] Alloxan is

in the plasma ascorbic acid [339]

Lactic acid is stated to accumulate in the muscles of animals deficient in ascorbic acid [254] Crandon [68], who induced experimental scurvy in himself, noted impairment in ability to perform aerobic work, e g running, jumping but anaerobic work e g working an ergograph with the fingers or forearm was unaffected

Much of the literature on ascorbic acid and carbohydrate metabolism is

confusing and needs careful interpretation Experimental work has sometimes been done on animals synthesizing ascorbic acid, e g the rat, sometimes on animals needing an exogenous supply, e g the guinea pig Observations on humans have been made on subjects whose state of ascorbic acid nutrition was unknown or on diabetics who recently came under control Tolerance of diabetics often improves with hospitalization Changes in blood sugar following the administration of ascorbic acid do not necessarily show that the latter affects carbohydrate metabolism Any changes may be due to alteration of renal threshold or to a temporary effect on storage mechanism

Interrelationship with Other Vitamins *Vitamin A* Sure and his co workers [273] showed that depletion of vitamin A in the rat is followed by a fall in the concentration of ascorbic acid in the tissues Pirie and Wood [341] also demonstrated a fall in the ascorbic acid content of the aqueous humour, and Mayer and Krehl [345] state that one of the first symptoms of vitamin A deficiency is depletion of the animals' ascorbic acid reserves as evidenced by scorbutic symptoms curable by ascorbic acid Mapson and Walker [349] while they agree that scorbutic symptoms appear in animals on diets deficient in vitamin A, attribute them not to ascorbic acid deficiency, but to restriction in total food intake It is also stated that ascorbic acid prevents the appearance of symptoms of vitamin A deficiency [352]

Aneurine A diet low in aneurine is stated to cause a temporary lowering of plasma ascorbic acid [351] Large doses of aneurine cause toxic effects [illegible] [353] If rats are made deficient in aneurine, ascorbic [illegible] ases the survival time of scorbutic guinea pigs [354]

Folic Acid and Vitamin B_{12} See p 161

Vitamin P Rutin, a compound with a vitamin P like action (p 732), increases the apparent biological action of ascorbic acid when this is supplied in sub maximal amounts [355] The mechanism of this action is uncertain, but it could be explained by the protective effect of rutin preventing the oxidation of ascorbic acid Papageorge and Mitchell [358] state that rutin has a "sparing effect" on adrenal ascorbic acid They attribute this to the anti oxidant action of rutin on ascorbic acid and adrenaline, the latter when oxidized accelerating the oxidation of ascorbic acid

Absorption of Ascorbic Acid Ascorbic acid is absorbed from the small intestine by a simple diffusion mechanism, the rate of absorption varying directly with the concentration of the ascorbic acid ingested [284] In the rat about sixty per cent of the ingested ascorbic acid is absorbed If large portions of this organ are resected absorption of the vitamin is inadequate [332] The degree of absorption depends on the concentration in the intestinal contents, rather than on the concentration in the blood tissues [957]

Certain strains of bacteria, including *B coli*, can decompose ascorbic acid *in vitro* [278], although whether they exert any destructive action in the gut is open to question Glucose has a protective action and may inhibit their action *in vitro* [280] A number of pathogenic enteric organisms e g *Salmonella, E typhosus Proteus morgagni*, can decompose ascorbic acid and it is conceivable that in pathological gastro intestinal conditions they may prevent adequate absorption [959] Gould and Shwachman [960] conclude that about twenty seven per cent of ingested ascorbic acid is destroyed somewhere in the gastro intestinal tract Melnick, Hochberg and Oser [363] however, state that nearly ninety-nine per cent of an oral dose of 200 mg of ascorbic acid is absorbed Ascorbic acid oxidase in foods is probably rapidly destroyed in the gastro intestinal tract

Farmer and his co workers [284, 285] believe that in man the absorption of ascorbic acid from the intestine is almost complete in health, because on normal intakes the fæcal excretion is negligible, less than 5 mg daily, and even on large doses of 1,000 mg daily does not exceed 15 mg

Under abnormal conditions intestinal absorption may be incomplete owing to the operation of certain factors interfering with it Abnormal bowel motility e g in diarrhœa and catharsis may be sufficiently severe to interfere with absorption even if large doses are given Abt and his co workers [287 831] have shown that there is tenfold increase in the fecal excretion of ascorbic acid in infants receiving sufficient magnesium sulphate to produce semi liquid stools Up to a quarter of the intake of ascorbic acid was found in the fæces

The absorption of ascorbic acid appears to be impaired in patients with achlorhydria [473] At any rate patients with this condition have abnormally low plasma ascorbic acid levels The administration of alkaline powders and alumina gel (aluminium hydroxide gel) does not interfere with the absorption of ascorbic acid [277 332]

Utilization of Synthetic Ascorbic Acid With the availability of vitamins in chemically pure form the question arose whether the synthetic form was utilized as well as the natural It has long been postulated that fruit and vegetables contain an unknown factor required for the utilization of ascorbic acid [367 881] In a recent investigation by Crampton and Burton [370] the authors reported that orange apple and tomato juice show a thirty five to forty five per cent higher ascorbic acid potency than is indicated by chemical assay The matter has been investigated by a number of workers [371 372 373 379] who have all shown that synthetic ascorbic acid is utilized just as well as the natural vitamin in vegetables and fruit According to Hollinger [380] some individuals utilize synthetic ascorbic acid better than ascorbic acid in green vegetables The availability of ascorbic acid in the latter is about eighty five per cent [383]

Storage and Distribution in the Body Ascorbic acid is found in all the tissues and fluids of the body About four fifths of the ascorbic acid intake is destroyed This occurs in the cæcum according to Reid [425] Muscle contains about 2 mg per 100 grams Glandular tissue particularly the adrenals is rich in the vitamin The adrenals contain more than any other organ or tissue (p 428) and in response to various factors the ascorbic acid level varies (p 429) There is also a high concentration in the intra ocular fluid ciliary body iris and lens [961 962] In the adult animal ascorbic acid probably enters the aqueous humour by a process of secretion the level is much higher (e g 50 mg per 100 ml) than in blood [364] Ascorbic acid is stored in those organs and tissues with a high metabolic activity Tumour tissue has a high ascorbic acid content [290] On exposure to cold there is a considerable increase in the ascorbic acid stored in the liver and kidneys [405]

Ascorbic acid is present in the blood C S F [297] saliva gastric juice [292] and milk The amount in C S F varies from 0 7 to 2 1 mg per 100 ml Strait and his co workers [307] obtained values twice this It may be increased from 1 8 to 4 1 mg after a 1 gram dose of ascorbic acid The amount in saliva is 0 07 to 0 25 mg per 100 ml and in gastric juice 1 05 mg per 100 ml when fasting and 0 9 mg after stimulation [292] There is no correlation between the amount in the saliva and gastric juice and in the blood and urine [292] This suggests that the secretion of ascorbic acid by the gastric mucosa and salivary glands is a physiological property of their cells

The blood ascorbic acid is subject to considerable variation particularly in the plasma The most recent figure for plasma ascorbic acid based on the examination of 110 normal subjects is 0 3 to 2 1 mg per 100 ml with an average of 0 98 mg [292] Other workers give a range of 0 6 to 2 5 mg per 100 ml [302] In some working class families the lower level might fall as low as 0 1 mg [300] As the plasma level varies with the intake a wide range of values has been reported from zero to 20 mg the latter being reached after the administration of large doses of the vitamin However the correlation is not absolute Thus Putnam and his co workers [294] noted that a daily intake of 32 mg among Mexican Indians maintained a plasma

level of 1 12 mg of ascorbic acid per 100 ml, while inhabitants of North Carolina had plasma levels below this on an intake of nearly 150 mg daily

The major part of the ascorbic acid is in the cells not the plasma Sargent [385] has determined the partition of the blood ascorbic acid between whole blood, plasma and the red blood cells The ratio of ascorbic acid in the plasma and cells is given by the equation $A_p = A_c - 0\,45$, A_p and A_c being the concentration of ascorbic acid in plasma and cells respectively expressed in mg per 100 ml According to Roe, Kuether and Zimler [388] and Daubenmerkl [397] the distribution of ascorbic acid between the plasma and the whole blood is related to the level of ascorbic acid in the blood They state that at whole blood levels of ascorbic acid below 0 6 mg per 100 ml the plasma content is lower than the whole blood content, at whole blood levels of 0 6 to 0 9 mg
as in whole blood, an
tent is higher than the
concentration of ascorbic acid in the cells is consistently greater than that in the plasma but he did not consider the wide range of blood values of Roe and his co workers According to Butler and Cushman [590] the average amount of ascorbic acid in the platelets and white cells is 34 mg per 100 ml, with a range of 29 to 43 mg An M R C report [407] gives the figure 16 6 mg per 100 ml on a daily intake of 20 mg of ascorbic acid The ascorbic acid is combined with the serum albumin in plasma [385 396] In patients suffering from serious illnesses the ascorbic acid in the plasma is lower than that in the whole blood [388] Thus both dietary intake and underlying pathological processes operate to produce the resulting pattern of distribution of ascorbic acid in the blood

Wilson and Lubschez [408] correlated the intake of ascorbic acid with the plasma and white cell layers They give the following figures

Daily Intake	Plasma Ascorbic Acid mg per 100 ml	Ascorbic Acid in White Cell Layer mg per 100 ml
0 5–1 9 mg per kilo	0 4 mg	20 mg
1 5–2 9 mg per kilo	0 7 mg	25 mg
9 mg per kilo	1 4 mg	25 mg

Thus, beyond a certain intake there is no increase in the ascorbic acid of the white cells It would appear that intakes of much more than 2 mg per kilo daily are normally quite unnecessary Wilson and Lubschez found that during convalescence from intercurrent febrile illnesses intakes of 3 to 9 mg per kilo were necessary to maintain white cell levels of 25 mg per 100 ml or more

Dodds and Macleod [312] correlated the plasma ascorbic acid with the daily intake in forty one adults of mean weight 57 kilo The wide range of values indicates that any estimation of the ascorbic acid intake based on plasma content would be quite fallacious

Daily Intake	Plasma Ascorbic Acid mg per 100 ml	Range mg per 100 ml
32 mg	0 48 ± 1 37	0 34–0 62
57 mg	0 72 ± 0 21	0 51 0 93
82 mg	0 93 ± 0 196	0 74–1 13
107 mg	1 05 ± 0 170	0 88–1 22

There is a seasonal variation in the blood ascorbic acid, the highest levels coinciding with the highest intake, i e in August [113] It is lowest in April

It has been stated that in pregnancy and lactation the blood ascorbic acid is lower than normal [305, 402], but Fredrikson [276] found no evidence for this, or for the statement that the blood ascorbic acid varies according to the stage of pregnancy, independent of seasonal variations The ascorbic acid content of fœtal blood is considerably higher than that of the maternal blood [276 406] Hamil and his co workers [406] found that the umbilical cord blood ascorbic acid ranged from 0 66 to 2 18 mg per 100 ml, the corresponding figures for the blood ascorbic acid of the mothers was 0 04 to 1 19 mg There is a decline in the blood ascorbic acid in the first week post partum The whole blood ascorbic acid is higher in males than females [411]

Ascorbic acid is secreted in the milk, the content of which depends on the mother's intake Surveys in Britain and Australia show that the average ascorbic acid content of fresh mother's milk is about 3 5 mg per 100 ml [414 416] with a range of 3 2 to 3 8 mg [415] A survey made in Chicago gave the figure 5 mg per 100 ml [419] Colostrum contains the most ascorbic acid (… lactation proceeds Th … the time of the year, … 00 ml in March to 5 1 …] found a mean daily intake of 5 9 mg in a group of women they studied in 1944 The output in the milk was 3 2 to 3 8 mg per 100 ml

Excretion of Ascorbic Acid Ascorbic acid is excreted in the fæces, urine and sweat The fæcal excretion is normally of the order of 5 mg daily but may be increased in diarrhœa and gastro intestinal disorders (p 223) The loss in the sweat is negligible, even under conditions of profuse sweating, when it varies from 0 8 to 2 7 mg daily [964–966] Much of this is in the form of dehydroascorbic acid

The urinary excretion of ascorbic acid depends on the intake [313] If large supplies are administered to persons whose stores are low the urinary excretion does not rise to normal levels at once The excretion remains low until the blood and tissues have taken up much of the ascorbic acid When this has occurred the blood and tissues are "saturated" * On an approximately constant water intake there is no correlation between the volume of urine and the excretion in the urine, there is only correlation between urinary volume and urinary excretion when the fluid intake alternates from day to day between widely different levels [964] Excretion is not affected by diuresis [550] Ascorbic acid like glucose is considered to be a 'threshold' substance The renal threshold that is the level at which ascorbic acid leaves the blood and passes through the kidney into the urine, has been calculated by giving supplements of … which there is a sudden sharp ris … 0 to 1 4 mg per 100 ml of pl … tates that it may be much lower, e g 0 85 mg per 100 ml, in a state of severe ascorbic acid deficiency, and Friedman and his co workers [319] consider that ascorbic acid is excreted in the urine at all plasma levels The renal threshold is a relatively stable figure for any one individual and varies little [430]

Excretion does not follow degree and rate of absorption but depends on the rate of absorption by the tissues The excretion never equals the amount ingested even when the body is saturated, as some is destroyed in the tissues Half to two thirds of the ingested ascorbic acid is metabolized to dehydroascorbic acid some of which undergoes irreversible destruction to diketogulonic acid and oxalic acid [865] Dehydroascorbic acid and diketogulonic acid are found in all tissues, the ratio of the former to ascorbic acid is increased in scurvy [1032] In man dehydroascorbic acid and di

* Th…

ketogulonic acid are excreted in approximately equal amounts, and together form about twenty per cent of the "total ascorbic acid" excreted [1031] Using ascorbic acid containing radio active C^{14}, it has been shown that in guinea pigs sixty six per cent of an ingested dose is broken down and expired as carbon dioxide and twenty two per cent is eliminated in the urine [798] According to Basu and Ray [322] there is no proportional relationship between average excretion and the state of "saturation unless the excretion is about 30 to 40 mg daily The excretion varies enormously in health and disease and, unless exceptionally low, is of little significance (see p 441) The daily excretion may be less than 5 mg in infants, and in the adult may vary from almost zero to 50 mg daily The common range of excretion is between 10 and 50 mg daily [240, 314, 375] Small amounts of dehydro ascorbic acid are excreted in the urine [905], the ratio of this to ascorbic acid varying from 0 05 to 0 4. The urinary excretion of ascorbic acid is lower in the winter months owing to the lower intake during this season Excretion is not influenced by menstruation [438]

If large doses of ascorbic acid are administered, e g 10 mg per kilo or 600 mg after a response has occurred then ninety four to ninety eight per cent of the daily excretion is excreted in the first thirteen hours, and seventy eight to eighty eight per cent in the first seven hours [800] Not more than fifty to sixty per cent of the ascorbic acid administered can be recovered from the urine when full saturation is reached on this dose [800]

The mechanism of the excretion of ascorbic acid by the kidney has been studied by Ralli [317] and her colleagues Studies on the simultaneous ascorbic acid and creatinine urinary clearance in the dog and ascorbic acid and inulin clearance in man showed that ascorbic acid is excreted in the urine by a process of filtration and active tubular reabsorption This view is supported by the observation of Leblond [318] that the concentration of ascorbic acid in the capsular fluid of the frog is the same as that of the plasma The reabsorptive mechanism for ascorbic acid appears to be limited to a maximal rate, so that when the vitamin is presented to the tubules by the glomerular filtrates at a rate exceeding this maximum, the excess is excreted in the urine The excretion of ascorbic acid in a given individual therefore, depends on (a) the blood plasma level (b) the rate of glomerular filtration and (c) the maximum rate of tubular reabsorption In a later paper Ralli [319] has shown that the ascorbic acid excretion in the urine does not become zero even with very low concentrations of ascorbic acid in the plasma but ultimately falls to a minimum and constant value and is then independent of the plasma concentration At lower excretion levels the excretion of ascorbic acid does not depend on the concentration in the blood nor on the filtration rate through the kidneys, but on the rate of tubular reabsorption which is extremely variable [317 319]

Any condition which reduces renal function impairs the excretion of ascorbic acid and may, therefore, falsify any conclusion regarding the level of ascorbic acid nutrition when this is based on excretion tests Sendroy and Miller [240] compared the urinary excretion of patients suffering from renal diseases with that of normal individuals They found that an abnormally slow excretion of a test dose of ascorbic acid does not necessarily indicate a low level of the vitamin in the body, because renal damage retards excretion even when the intake of ascorbic acid is adequate The effect of decreased kidney function on the ascorbic acid clearance was found to run parallel to the effect on the urea clearance Similar findings are reported by Wright and his co workers [550, 576] No appreciable destruction of ascorbic acid occurs in the urine stored in the bladder [321]

Many foods, drugs and external conditions influence the excretion of ascorbic acid Diets high in protein cysteine and methionine accelerate excretion in the rat [439, 504 980], glucose decreases tubular reabsorption in the dog and leads to an increased excretion [441] Body rest and posture

have no effect Conditions of stress, such as exposure to cold [405] burning hæmorrhage, muscle trauma and severe exercise [443], lead to increased excretion (p 448) Under stress the excretion may be greater than the intake [310], due to mobilization of ascorbic acid from the adrenals, which become depleted There is a decreased excretion during the healing of wounds [923] and in patients recovering from severe injury, hæmorrhage and infection [446] The excretion of ascorbic acid is markedly decreased by exposure of human subjects to the anoxic conditions of an altitude of 18 000 feet [974], at an altitude of 35,000 feet and breathing one hundred per cent oxygen there is increased excretion [799] The observation that ascorbic acid increases the altitude tolerance of mice is of interest in this connection [975] Pyrexia *per se* probably has no effect on ascorbic acid excretion (p 448)

Various drugs affect the excretion of ascorbic acid Insulin [247] ammonium chloride [324, 449], salicylates [450] and anti histamine drugs [330] increase the urinary excretion of ascorbic acid So do thiouracil [168] thyroid 2 4 dinitrophenol [994], local anæsthetics [915], atropine, aspirin, cincophen, barbiturates, amidopyrine adrenaline chloroform chloretone, paraldehyde stilbœstrol œstradiol and sulphonamides [168, 324, 325, 327, 328] Sodium bicarbonate causes a lowered urinary excretion of ascorbic acid [449] The urinary excretion of ascorbic acid is markedly increased under hot and moist environmental conditions compared with normal or hot and dry conditions [964]

Increased and decreased absorption of ascorbic acid due to drugs or other factors is not necessarily due to changes in the utilization of the vitamin, nor does it indicate that requirements are increased Drugs may change renal tubular absorption e g the œstrogens [327] or they may alter the permeability of the kidney, or ascorbic acid might be mobilized from the adrenals or white blood cells The salicylates increase the excretion of ascorbic acid by depleting the ascorbic acid in the adrenals [502]

HUMAN REQUIREMENTS OF ASCORBIC ACID

Requirements based on Dietary Studies The human requirements of scorbic acid have been determined by observing the minimum intake needed prevent the appearance of scorbutic or deficiency symptoms and from etary surveys The minimum amount that prevents scurvy may not be fficient for the maintenance of good health Thus Stepp and Schroder [346] nd that the dose of ascorbic acid preventing scurvy in guinea pigs does not vent dental lesions Perhaps some idea of the ascorbic acid requirements be obtained from a study of the infant which receives some 40 to 50 mg y in its milk provided its mother is healthy and well nourished On the er hand Nansen, the explorer, lived on a meat diet for nearly a year, and this could not have supplied much more than 10 mg daily, yet he kept in good health The synopsis overleaf gives some of the methods used for assessing human ascorbic acid requirements based on dietary studies and the daily intake suggested

Certainly a very low intake of ascorbic acid appears to be compatible with good health Meat and fish eating races such as the Eskimos probably consume no more than 20 mg daily Fox [347] kept a prisoner for ten months on a diet of cooked meat, which could not have supplied much more than 10 mg daily Although he had manual work to do he showed no deficiency symptoms, the urinary excretion was about 1 mg daily

A very thorough investigation was carried out by the Accessory Food Factors Committee of the Medical Research Council between 1944–46 to determine the ascorbic acid requirements of the human adult [407] Twenty subjects aged twenty one to thirty four were given a diet adequate in all respects except that it supplied only 1 mg ascorbic acid daily One group

Author	Method	Suggested Daily Intake of Ascorbic Acid
Rietschel and Mensching [342]	Smallest intake compatible with health and absence of scurvy	10 15 mg
Medical Research Council [407]	Prevention and cure of scurvy	10 mg
Kalk and Bruhl [384]	Daily dose required to cure scurvy	10 mg
Pijoan and Lozner [873]	Minimum daily intake compatible with good health	15 mg
Leeson, H J, *et al*	Minimum daily intake compatible with good health	25 mg
Fox *et al* [344]	Minimum daily intake compatible with good health	15 mg
Fox *et al* [347]	Diet of cooked meat	? Probably 10 mg
Crandon *et al* [C8]	Daily consumption necessary for re excretion of ascorbic acid in urine after treatment of scurvy	30–45 mg
Johnson, *et al* [835]	Saturation" after two months deprivation of ascorbic acid	75 mg
Najjar, *et al* [343]	Daily intake that did not produce deficiency symptoms over period of eighteen months	25 mg
Kyhos, *et al* [386]	Cure of gum lesions in prisoners with zero ascorbic acid in plasma	50 75 mg

received no supplementary ascorbic acid, another received 10 mg daily, and another 70 mg daily The group receiving no ascorbic acid developed scorbutic symptoms after seventeen to twenty one weeks, characterized by hyperkeratosis of hair follicles, perifollicular hæmorrhages, hæmorrhages of the gums, delayed wound healing and ecchymoses of the limbs These symptoms were cured by 10 mg of ascorbic acid daily, the results were no better with 20 mg daily No scorbuti

a group of volunteers receiving 10 mg

and twenty-four days, and they rem

ascorbic acid was less than 0 05 mg per 100 ml, and white cell ascorbic acid content was between 1 5 and 3 mg per 100 ml The scorbutic group had a plasma ascorbic acid of zero to 0 03 mg per 100 ml and a white cell level of 1 mg or less per 100 ml About one hundred days elapsed between the disappearance of ascorbic acid from the plasma and the first clinical signs of scurvy

Although a daily intake of 10 mg protects against scurvy, this is a marginal figure and to provide a safety margin it is probably wise to double or even treble this figure Tests on physical fatigue leave some doubt whether 10 mg daily is optimal [407] Thus Hagtvet [409] noted scorbutic symptoms in polar explorers doing heavy work on ascorbic acid intakes of 5 to 15 mg daily Fox [347], however, observed no deficiency symptoms in a native doing heavy work on an intake of 10 mg daily

Dietary surveys of the population have been made To get satisfactory results the ascorbic acid must be assayed in the food as it is presented at the table and not before or just after cooking The diet of an individual or a group must be examined for a week and representative samples analysed Values calculated from food tables will give highly fallacious results

Author	Class of Subjects Examined	Daily Intake of Ascorbic Acid in Food in mg
Widdowson and Alington [833]	'Middle class'	57 mg in 1935; 27 mg in 1941
McNee and Reid [971]	Royal Navy and civilians	15 mg
Stuhl [972]	British soldiers	>25 mg
Stamm *et al* [1042]	R A F personnel	17–26 mg
Ungley [973]	Navy personnel	16–30 mg

No deficiency symptoms were observed on low intakes of 15 mg of ascorbic acid daily

Requirements based on a Study of Capillary Fragility. In 1931 Gothlin [348] stated that capillary fragility is a measure of the level of ascorbic acid nutrition, and he employed it to determine the human requirements of the vitamin From simultaneous determinations of capillary fragility tests and blood ascorbic acid levels Gothlin stated capillary fragility becomes pathological at plasma levels of 0 1 to 0 14 mg ascorbic acid per 100 ml He put the minimum human daily requirements of ascorbic acid at 20 to 30 mg for a 60 kg subject More recent work suggests that the determination of c (p 415) I depriva t resistance [407]

Requirements based on Examination of Urinary Excretion of Ascorbic Acid Many workers have attempted to find the human ascorbic acid requirement from studies on the urinary excretion of the vitamin [348, 356, 357] An arbitrary excretion figure, e g 25 mg, has been adopted and the daily intake found that produces an excretion in excess of this This is considered to be the daily requirement The excretion of ascorbic acid is not a measure of the adequacy of the intake An almost zero excretion is compatible with good health (p 438) Even if an arbitrary excretion figure is accepted as a measure of adequate intake excretion is affected by so many factors that it fails to be an accurate measure of intake The renal threshold, for example, is exceedingly variable and may fall between 0 85 and 1 4 mg per 100 ml Deeny and his collaborators [970] have shown that marked hourly variations in excretion occur at all physiological levels of intake and that they are independent of the rate of flow of the urine

"Saturation" or 'loading" tests have been devised to assess ascorbic acid requirements in place of simple excretion studies The technique of the test depends on the hypothesis that after the administration of a given dose of ascorbic acid the tissues and organs must first be "topped up" with an adequate supply before the blood level rises to the renal threshold so that increased quantities are excreted through the kidneys in the urine Briefly the method consists of determining the intake that causes a sharp rise in the urinary excretion (p 467) The authors see no reason why "saturation" with ascorbic acid should be essential for good health although many workers believe it is The values obtained by saturation tests vary from 30 to 100 mg daily as shown in the following table

Author	Daily Ascorbic Acid Requirement
van Eekelen, 1936 [365]	60 mg
Schnetzer, 1937 [369]	40 mg
Widenbauer, 1937 [376]	30–50 mg
Heinemann, 1938 [368]	0 8 mg per kilo (= 50 mg)
Kellie and Zilva, 1939 [359]	30–50 mg
Hauck *et al*, 1939 [374]	1 1 6 mg per kilo (= 65 100 mg)
Todhunter and Robbins, 1940 [375]	1 6–1 7 mg per kilo (= 100 mg)
Lewis *et al*, 1943 [976]	75 mg
Kline and Eheart, 1944 [377]	1 4–1 8 mg per kilo (= 75 100 mg)
Haines *et al*, 1947 [366]	70 mg

Hauck and her associates [374] first saturate the individual with 200 mg ascorbic acid daily for six days saturation is confirmed by an increase of excretion following a test dose of 400 mg The test is repeated two or three times Finally a series of similar tests is conducted on graded doses of ascorbic acid and the smallest amount found that will induce a similar response to the test dose of 400 mg as obtained in the preliminary tests This is the maximum requirement to maintain tissue saturation Todhunter and Robbins [375] gave the subject a basal diet furnishing 20 mg of ascorbic acid daily and the response in urinary excretion to a 400 mg test dose following a four day period when 200 mg daily was taken was determined for three successive periods Widenbauer [376] gave the individual under test a standard diet containing little ascorbic acid for several days and calculated the average daily excretion This served as a preliminary blank Test doses of 200 to 500 mg of ascorbic acid were then given until saturation was reached this was arbitrarily assumed to be reached when at least fifty per cent of the last test dose was excreted in twenty four hours or half this in twelve hours The daily dose was then adjusted to give an excretion of ascorbic acid slightly higher than the preliminary blank This dose was given for seven days the urine being titrated daily to find the average excretion The daily requirement was found by taking from the average daily intake during the final period the average daily excretion during the same period less the average daily excretion of the preliminary period A similar method was employed by Lewis and her co workers [976] Figures for the human requirements of ascorbic acid based on excretion and saturation tests are based on purely arbitrary assumptions Both the size of the test dose given and the criterion of adequate excretion vary from one worker to another It has not been proven that an individual in a state of ascorbic acid saturation is any healthier than one who is not

Requirements calculated from Blood Levels Ascorbic acid requirements based on plasma blood levels must be accepted with considerable caution (p 469) The range of so called normal values is wide Many observers believe that plasma ascorbic acid values below 0 5 to 0 7 mg per 100 ml indicate ascorbic acid depletion and that values of 0 7 to 1 mg per 100 ml indicate mild deficiency Purinton and Schuck [988] consider 0 8 mg per 100 ml indicates an adequate intake Ralli and her associates [378] obtained the mean value 1 2 mg for a group of students Isolated blood plasma figures are of little value Dodds and Macleod [997] and others [294] have shown that there is no consistent relationship between plasma ascorbic acid levels and intake Dodds and Macleod brought their subjects into equilibrium or slight ascorbic acid deficiency as shown by plasma ascorbic acid averages which slowly decreased or were just maintained This was accomplished by an initial intake of 32 to 35 mg daily Then the intake was gradually increased by large test doses over periods of eight to ten weeks and plasma levels found An intake of 1 mg per kilo (60 mg for a 60 kilo subject) increased plasma ascorbic acid for all subjects studied and this was considered to represent the daily requirement On the other hand Wright and his co workers [404] found supplements of 25 mg of ascorbic acid a day sufficient to maintain whole blood or plasma values when human subjects were on a diet containing no ascorbic acid Bessey White [858] Horwitt [859] and others [861] gave supplements of ascorbic acid until there was a rise in the plasma level this corresponded to 50 mg daily Fincke and Landquist [977] arbitrarily fixed a plasma level of 0 8 mg per 100 ml as an index of adequate intake and found that 40 to 90 mg of ascorbic acid daily was necessary to maintain this Goldsmith and her associates [551] considered the optimum plasma level to be 1 mg per 100 ml and found that an intake of 70 mg daily provided for this

It is now established that the plasma ascorbic acid level unless near zero is of little value in assessing the ascorbic acid reserves of the body Blood

plasma levels of 0 02 mg per 100 ml have been recorded in the absence of scurvy [367] According to a Medical Research Council report [407] a plasma value below 0 1 mg per 100 ml indicates a daily intake of about 20 mg

The ascorbic acid of whole blood or the white cell and platelet layer is of far greater significance than the plasma level (p 469) This can now be done on 0 1 ml blood [413] Whole blood ascorbic acid values reflect the tissue reserves of the vitamin plasma values represent an overflow Owing to the technical difficulties involved in the estimations very few determinations have been made in conjunction with dietary studies or nutrition surveys

Wilson and Lubschez [408] have shown that to maintain a level of 25 mg per 100 ml of ascorbic acid in the white cell layer which is considered an optimal level since it will not rise much above this whatever the intake an intake of 100 mg ascorbic acid daily is necessary They consider that daily intakes above 200 mg unnecessary and inadvisable

Correlation of Ascorbic Acid Levels in Blood and Urine According to Faulkner and Taylor [314] plasma ascorbic acid values above 1 4 mg per 100 ml the renal threshold correspond to a state of saturation and lower values correspond to an unsaturated state The renal threshold however has a very wide range from 0 85 to 1 4 mg per 100 ml [68 314 430 967] This makes such a test valueless Roberts and co workers [981] determined blood concentrations and the urinary response to a test dose on intakes ranging from 32 to 82 mg of ascorbic acid daily A blood level of 0 7 mg per 100 ml and an excretion of fifty per cent of a 300 mg test dose in twenty four hours were arbitrarily used as criteria indicating a satisfactory intake On these criteria 62 to 72 mg daily was considered adequate for girls of six to twelve years

Ralli and her co workers [378] argued that the amount of ascorbic acid required daily would be the smallest amount necessary to maintain a normal plasma level At this level the amount excreted should be small and should remain fairly constant so that the maximum capacity of the kidney tubules would be exceeded Then if more than the daily needs were given excretion should rise promptly In the technique used by Ralli the subject is given a diet containing 5 mg of ascorbic acid daily The twenty four hour ascorbic acid excretion and the plasma level are determined and then graded intakes of ascorbic acid given On an intake of 50 mg daily for one hundred and twenty seven days the plasma ascorbic acid averaged 0 4 mg per 100 ml Increasing the intake of ascorbic acid did not alter the amount excreted as long as the intake was below 100 mg daily At this intake there was a sharp rise in the urinary excretion and a plasma level of 1 mg per 100 ml It was therefore concluded that to maintain tissue saturation and a plasma level of 1 mg per 100 ml the daily intake of ascorbic acid must be at least 100 mg daily This was considered to be the optimum daily requirement

Haines and her co workers [366] placed groups of healthy adults for six weeks on diets providing 30 53 and 70 mg ascorbic acid daily after preliminary saturation with 400 mg of ascorbic acid At the end of six weeks the tissue reserves of ascorbic acid were judged by fasting plasma ascorbic acid values Intake was considered adequate if the fasting plasma levels reached a pleateau and if fifty per cent of a test dose of 400 mg of ascorbic acid was excreted in twenty four hours A daily intake of at least 70 mg of ascorbic acid was considered necessary to satisfy these criteria

Purinton and Schuck [988] consider that a plasma level of 0 8 mg or more per 100 ml indicates an adequate level of ascorbic acid nutrition To determine the human requirements they measured the ascorbic acid plasma concentration and urinary elimination of [illegible]
response to a test dose of 500 mg The s [illegible]
14 to 15 mg of ascorbic acid and fasting [illegible]
Then the test dose was given orally or intravenously and the plasma ascorbic acid measured at regular intervals until the absorption of the dose was indi

Author	Method	Age Group and Daily Requirement of Ascorbic Acid
Iverson and Daniels 1936 [421]	Graded supplements given until sudden rise in excretion occurred	Under 5 years 120 mg or 7 mg per kilo
Widenbauer 1937 [376]	Oral test doses until saturated (50% of test dose excreted in 24 hours)	3 years 22 mg
Bessey and White 1942 [858]	Dose of ascorbic acid needed to produce rise in plasma level	5 13 years 40 50 mg
Roberts *et al* 1943 [981]	Blood concentrations (over 0 7 mg per 100 ml) and urinary response to test dose (50% excreted in 24 hours)	6 12 years 62 72 mg
Meyer and Hathaway 1944 [445]	Saturation test	Under 5 years 25 mg
Storvick et al 1949 [382]	*Intake needed to maintain a plasma ascorbic acid of 0 8 mg per 100 ml*	*Girls 16–19 years 70 mg* boys 18 years 90 mg
James 1943 [982]	Analysis of ascorbic acid in diet	Schoolchildren 10 15 mg
Booth *et al* 1942 [984]	Analysis of ascorbic acid in diet	Schoolchildren 15 mg
Widdowson and McCance 1942 [996]	Analysis of ascorbic acid in diet	Public schoolboys 15 20 mg schoolchildren 32 mg
Harris and Olliver 1943 [893]	Analysis of ascorbic acid in diet	Inmates of children s home 19 24 mg spring and winter 24–55 mg summer and autumn

Recommended Daily Allowance of Ascorbic Acid

National Research Council U S A

	Calories	Ascorbic Acid mg
Man (154 lb 70 kilo)		
Sedentary	2 400	75
Physically active	3 000	75
With heavy work	4 500	75
Woman (123 lb 56 kilo)		
Sedentary	2 000	70
Moderately active	2 400	70
Very active	3 000	70
Pregnancy (latter half)	2 400	100
Lactation	3 000	150
Children up to 12 years		
Under 1 year	110 per kilo	30
1–3 years (27 lb 12 kilo)	1 200	35
4 6 years (42 lb 19 kilo)	1 600	50
7 9 years (58 lb 26 kilo)	2 000	60
10 12 years (78 lb 35 kilo)	2 500	75
Children over 12 years		
Girls		
13 15 years (108 lb 49 kilo)	2 600	80
16 20 years (122 lb 55 kilo)	2 400	80
Boys		
13 15 years (108 lb 49 kilo)	3 200	90
16–20 years (141 lb 64 kilo)	3 800	100

(p 440) show that not many individuals in Britain at any rate, receive the intake of ascorbic acid that is considered optimal according to laboratory tests

National Research Council (U S A) Recommendations In 1941 the Committee on Food and Nutrition of the National Research Council drew up a table of the probable daily requirements of various vitamins These were tentative figures for use in planning dietaries and allowed for a considerable wastage in the preparation of food The values were revised in 1945 and 1948 Those for ascorbic acid are given on p 446

The Nutrition Committee of the British Medical Association (1950) recommended a much lower daily intake—30 mg daily for adolescents of both sexes 20 mg for an adult male irrespective of occupation, 10 mg for children up to one year old, 15 mg for children two to six years and 20 mg for children over seven years

Requirements and Age The ascorbic acid requirements are probably related to basal metabolic rate [988] so that adolescents particularly boys require more than subjects in any other age group It has been stated incorrectly that requirements are increased in old age because the subjects tested had a low excretion or showed increased retention on test dosing [452 683] The probability is that they were judged by standards of young adults and their food intake including that of ascorbic acid was much lower than normal The subjects were hospital patients and not normal subjects

Requirements in Pregnancy and Lactation During pregnancy there is a lowered excretion of ascorbic acid [417] and blood plasma levels fall progressively right down to term [390 442 989] This would be expected as ascorbic acid is stored by the fœtus at the expense of the mother (p 444) Neuweiler [417] found that it was necessary for pregnant and nursing women to ingest larger test doses of ascorbic acid than normal subjects to maintain the same level of excretion Using saturation tests (p 471) various workers have calculated the daily requirements of pregnant and nursing mothers [376 410 414 420 423] The values fall between 33 mg and 100 mg It is not surprising that the plasma ascorbic acid and the urinary output should be low in the lactation period the ascorbic acid is secreted into the milk and not excreted in the urine The work of Munks and her colleagues [419] demonstrated the wide variations in ascorbic acid metabolism among normal lactating women and the unreliability of accepting levels of the vitamin in the blood and urine as an index of adequate intake The amount excreted in the urine bore no relation to the intake or that present in the milk Intakes of 100 mg ascorbic acid daily were often associated with a low urinary excretion and fasting blood levels were frequently as low as those considered by some authorities to indicate scurvy (e g 0 1 mg per 100 ml) A low blood and urine ascorbic acid did not seem to have any deleterious effect on mother or child When supplements of ascorbic acid were fed the bulk appeared in the urine and not the milk [392] There is an upper limit or threshold value about 7 to 8 mg per 100 ml which cannot be exceeded whatever the mother s intake of ascorbic acid [418]

To supply the infant with 15 to 30 mg of ascorbic acid daily it would seem that during lactation the mother s intake should be at least 45 to 60 mg daily This can normally be obtained from a good diet The requirements recommended by the National Research Council (1948) are 100 mg daily in pregnancy and 150 mg during lactation These quantities are certainly very generous and there can be very few women in Britain who obtain such in intake The Committee on Nutrition of the British Medical Association (1950) suggested 40 mg daily in pregnancy and 50 mg during lactation It should be possible to obtain enough ascorbic acid from a good diet without having resource to supplements of the synthetic vitamin

Requirements and Exercise Experimental evidence concerning the effect of ascorbic acid on exercise is conflicting Several worker

ported a decreased excretion after severe exercise, and diminished efficiency on low intakes of ascorbic acid [386, 443, 455, 462] Other workers claim that the administration of ascorbic acid increases efficiency and wards off fatigue [846, 990] Much of this work has been uncontrolled and not submitted to statistical examination Subjective statements by volunteers undergoing fatigue tests are difficult to interpret Johnson and others [835] have shown that two months' deprivation of ascorbic acid leads to no detectable deterioration in physical vigour, and that supplements of 75 mg of the vitamin daily do not produce any benefit in manual workers with respect to well being physical vigour and efficiency This work was controlled and evaluated statistically

It has not been proven that additional ascorbic acid is needed in severe exercise Low blood and urine figures could be explained by redistribution in the body, e g storage in the adrenals as part of a stress phenomenon (p 432) If the requirements of ascorbic acid are related to metabolic activity [988] one would expect them to be increased by exercise, although the best experimental evidence is against this

Faulty Absorption Some gastro intestinal conditions interfere with the absorption of vitamins (p 225) In such cases an increased intake is necessary to compensate for this

Effect of Drugs Many drugs alter the excretion of ascorbic acid but unless they affect general metabolism there is no evidence that they affect the normal requirements of the vitamin

Requirements and Infections A diminished excretion of ascorbic acid has been observed in a number of infections [119–127, 432–437] The plasma ascorbic acid is also lower than usual [123] The fall in plasma and urinary ascorbic acid is not due to the pyrexia *per se* but is associated with the infective process [992, 993] This fall is due to mobilization of ascorbic acid from the plasma to the white cells which become laden with ascorbic granules (p 420), and to the adrenals There is no direct evidence that infection increases the requirements of ascorbic acid The fall in plasma and urine levels are quite unreliable guides of adequacy of intake (p 441) Most workers, however, assume that requirements are increased

Requirements and Raised Metabolism A decreased excretion of ascorbic acid has been observed in patients with diseases characterized by increased [illegible] g malignant disease [440], leukæmia [474], and [illegible] e experimental animal drugs increasing general [illegible] 2 4 dinitrophenol and insulin increase the excretion of ascorbic acid [994] As requirements are related to metabolic rate, requirements are probably increased in the conditions mentioned

Requirements and Climate The ascorbic acid requirements are not raised by a high environmental temperature In tests carried out by Henschel and his associates [869, 879] the work performance of subjects at high environmental temperatures (122° F) was uninfluenced by high or low intakes of ascorbic acid It is not certain whether climate has any effect on ascorbic acid requirements Scurvy is a disease of temperate and not tropical climates This may be because native races consume more raw food than the inhabitants of temperate lands

DISEASES ASSOCIATED WITH ASCORBIC ACID DEFICIENCY

SCURVY

Ætiology and Incidence Scurvy is a deficiency disease considered to be due mainly to an insufficient intake or absorption of ascorbic acid It has been maintained that lack of this vitamin is not the sole causative factor, since cases of scurvy have been described resistant to treatment with pure ascorbic acid Some of these cases, however, responded to parenteral but not to oral treatment with the vitamin Mawson [444] has described a case

of scurvy in which the ascorbic acid intake was adequate although the excretion and the blood level were low. "Renal scurvy" was diagnosed implying that the patient had an abnormally low renal threshold for ascorbic acid and was therefore unable to store sufficient in the tissues to prevent scurvy. With the discovery of other vitamins in fruits, particularly vitamin P, it has been suggested that scurvy like ... pellagra is a multiple avitaminosis. According to Szent-Gy ... (pp 731–738) scurvy is a disease due to a combined ... ascorbic acid and vitamin P. Cameron and Mills [1015] ... vitamin P but not ascorbic acid to a case of classic scurvy. The hemorrh... promptly disappeared but the other manifestations were unalt... with the observation of Mouriquand and Edel [45... the administration of vitamin P precipitated hemorrh... scorbutic diet.

How long does it take for a healthy adult to ... on a diet otherwise adequate but devoid of ascorbic ... various answers to this question. According to ... and six months at sea on a scorbutic diet. Men ... poor in ascorbic acid for a hundred days without ... [?s] who lived on a diet adequate in all respects bu... observed that scurvy developed after a hundred ... total deprivation. Symptoms of scurvy appeare... refugees after three to eight months on a deficien... salt turnip [834]. In infants scurvy develops bet... month [514]. Farmer [864] observed no clinical evid... hyperkeratotic papules surrounding the hair follicles ... on a scorbutic diet (0 to 10 mg ascorbic acid daily) ... trial carried out in Britain under the M R C the first si... keratosis of hair follicles—appeared after seventeen to twen... after twenty six to thirty four weeks perifollicular hemorrh... thirty to thirty eight weeks swelling and bleeding of the gu...

Factors responsible for the actual appearance of scurvy in ... scorbutic diet are climate, composition of the food in relation to other vitamins and minerals, constitution of the subject, condition of internal secretions presence of infection, and the condition of the alimentary tract. According to Lund [460] patients with gastric lesions have low reserves of ascorbic acid and are often near the scorbutic level. The causes of scurvy met with in the population at large are "ignorance, apathy and poverty" [927]. According to McMilland and Inglis [927] there was an increase in scurvy during the last war. In 1941–42 scurvy formed over three per cent of all admissions to medical ... According to the Ministry o... Glasgow was ... Inglis att... ignorance ... n ("bachelor scurvy"), and to the need for potatoes and vegetables in the diet. Secondly to apathy because such foods require preparation and cooking and thirdly to poverty, making it impossible to buy an adequate diet. Pure scurvy, although common in the days when ships went to sea carrying their own food for months is now rare. It is often accompanied by signs of other vitamin deficiencies such as neuritis (aneurine), glossitis (nicotinic acid), and cheilosis (riboflavine).

Clinical Manifestations of Scurvy. The adult suffering from scurvy complains of tiredness and weakness and pain in the limbs. An early manifestation is the appearance of hyperkeratosis of the hair follicles, which is followed by perifollicular hæmorrhages, particularly on the legs, which are brawny and tender.

As navigators and explorers have observed weakness is one of the early complaints of scurvy. This is associated with fatigue on exertion, palpitation,

anorexia and breathlessness The patient sits, rather than stands or walks and when he does stand he flexes his legs Hæmorrhage from the gums and stomato gingivitis hypertrophy of the dental papillæ (see Fig 158) followed by loosening of the teeth are common symptoms of scurvy, the loosening of the teeth is due to the resorption of the alveoli of the jaw bones One of the most reliable early signs of scurvy is gingivitis The characteristic swollen boggy and tender gums of scurvy occur only when teeth are present and are most marked about defective teeth (Figs 158, 159) Not only may hyperæmia and hæmorrhage of the gums occur, but epithelial degeneration ulceration and even gangrene Owing to the infection around the base of the teeth the breath is fœtid Other hæmorrhagic manifestations include hæmaturia melæna with diarrhœa pin point hæmorrhages in the gut with subsequent ulceration menorrhagia metrorrhagia epistaxis and subperiosteal hæmatomata The latter cause the limbs to be painful to the touch Extravasations of blood from larger vessels into the muscles may occur (Fig 161), as well as beneath mucous membranes in the gums conjunctivæ and lining of the serous cavities and joints Peritoneal irritation may occur through extravasation of blood into the peritoneal cavity Hines [448] describes three cases of scurvy that presented as acute surgical disease of the abdomen Two were subjected to surgery and bleeding was shown to be the cause of peritoneal irritation Petechial hæmorrhages are also present (Fig 160). Purplish discoloration may occur around the joints abdomen face and popliteal fossa Farmer [864] observed petechiæ following slight trauma in volunteers kept on a scorbutic diet for five months The scleræ are slightly icteric

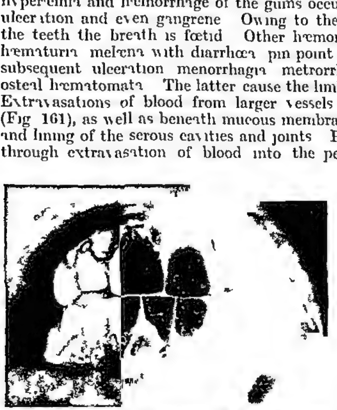


FIG 158 Scorbutic Gingivitis The patient is an orphan on the gums are clearly although irregular

The hæmorrhagic manifestations have been stated to be due to abnormal capillary fragility, although more modern work negatives any connection between ascorbic acid deficiency and increased capillary fragility (p 415) According to Lee and Lee [88] there may be a failure of the contractile mechanism of the small vessels resulting in their dilatation and a sluggish blood flow (Figs 162 165) Scarborough believes that vitamin P may play a part in the hæmorrhagic manifestations of scurvy (p 738) Only a third of all cases of scurvy show an increased capillary fragility [860]

The complexion is sallow, dirty yellowish grey and cadaveric and the extremities may be cold and cyanotic Long splinter like hæmorrhages are sometimes seen under the nails

The anæmia of scurvy is discussed on p 453 It is not a constant finding and conforms to no particular type It may be macrocytic normocytic or microcytic A mild hypochromic and normocytic anæmia is common In their cases Spies and his co workers [103] observed a persistent reticulocytosis and moderate leucopenia and thrombopenia The anæmia of scurvy is probably not due to ascorbic acid deficiency alone as a simple uncomplicated deficiency does not produce scurvy [68 407] and anæmia when it does occur sometimes responds to treatment with iron alone [110] Usually the serum iron is normal [103] The icteric index is 10 to 20, suggesting hæmolysis

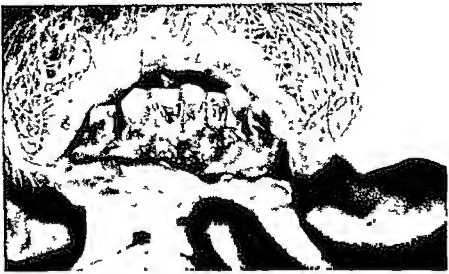
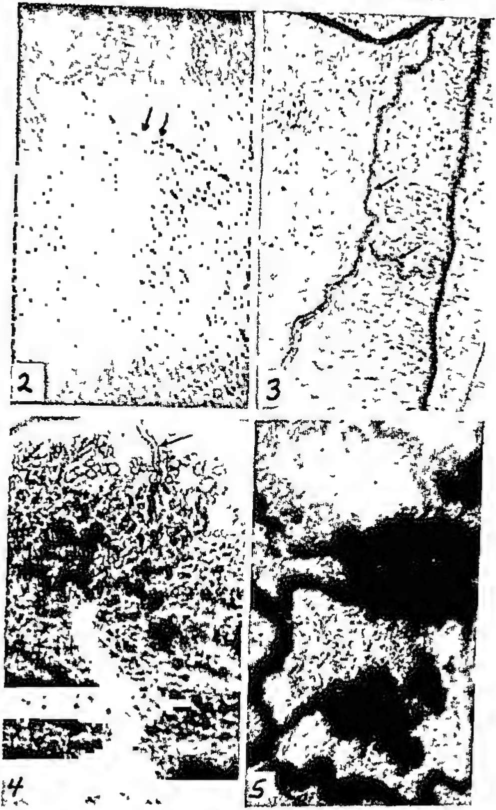


Fig 159 Scurvy A patient with scurvy showing considerable swelling sponginess and discoloration of the gums

Fig 160 Scurvy Forearm of the same patient as in Fig 159 showing bleb like extravasations of blood resulting from slight traumata a year previously Petechial hæmorrhages resulting from a recent tourniquet test are also seen

Fig 161 Scurvy The same patient as in Fig 159 showing extravasation of blood around right ankle and on right shin Some œdema of the feet is present



FIGS 162 to 165 The Peripheral Vascular System in Scurvy (approximately × 120)

FIG 162 (*upper left*) Normal animal arteriole in an environment of adrenaline, 1 in 5 million The lumen of the capillary (arrows) is empty, due to contraction under the influence of adrenaline

FIG 163 (*upper right*) Scorbutic animal a terminal arteriole in an environment of adrenaline 1 in 5 million Both capillaries (arrows) have failed to contract and are filled with a sluggish stream of blood

FIG 164 (*lower left*) Scorbutic animal small venules draining the capillaries are partly surrounded by small hæmorrhages, seen as dark masses in the centre of the figure At the bottom of the figure two large masses of blood are visible outside the main venule

FIG 165 (*lower right*) Scorbutic animal, showing larger petechiæ along the tributary branches of the collecting venules

as there are no signs of liver dysfunction [103]. Red cells may be found in the urine.

Infection with its resulting pyrexia is common. The depleted ascorbic acid reserves of the patient suffering from scurvy are said to render him more susceptible to infection. If death occurs it is due to intercurrent infection, e.g. bronchopneumonia, or it suddenly occurs with syncope. Crandon [68], however, contests these views. Although he suffered from frank clinical scurvy for a month and subclinical scurvy for several months he remained free from infection with the exception of colds.

Crandon [68] placed himself on a diet free from ascorbic acid but containing all the other essentials, including a complete range of all the other vitamins and minerals. During the first four months all the clinical findings were negative. There was a slight loss of weight, a fall in the metabolic rate and a feeling of weakness and fatigue on slight exertion. It was only after a hundred and thirty-four days had elapsed that clinical manifestations of scurvy appeared. The loss in weight, which eventually reached a maximum of 27 lb., was attributed to the monotonous diet rather than to the absence of ascorbic acid. The following description is taken from the paper by Crandon and his co-workers [68].

Skin Lesions. After a hundred and thirty-four days had elapsed small hyperkeratotic papules began to develop over the buttocks and posterior aspects of the calves; there was also noticeable fragmentation of hairs. These lesions resembled the follicular keratitis of vitamin A deficiency. Each papule contained an ingrown hair which could be seen if the hyperkeratotic plug was picked or scraped off, leaving a small slightly bleeding crater. There was also marked dryness of the skin, particularly over much of the extensor surfaces and backs of the hands. The existence of vitamin A deficiency was ruled out because 30 000 units of vitamin A were being taken daily and biophotometer readings and plasma vitamin A values were within normal limits.

After a hundred and sixty-one days small perifollicular hæmorrhages or petechiæ appeared on the legs, particularly after standing for long periods. After six months they were abundant over the whole of the leg and on the thighs took the place of the hyperkeratotic papules. The administration of ascorbic acid rapidly cleared these lesions.

Wound Healing. The experiments of Crandon and others on the healing of wounds have been previously described (p. 413).

Teeth and Gums.
in the teeth or gums
on pressure but no oth
showed normal tissue
the teeth showed occa
bleeding occurred on brushing the teeth.

Farmer [864] kept several volunteers on a scorbutic diet (0 to 10 mg. ascorbic acid daily) for five months but failed to observe any changes in the gums, teeth or jaws either with the naked eye, radiologically or with the biomicroscope. This period may not have been long enough (see M.R.C. report [407]).

Hæmatological Picture. Although blood was lost by venesection on four occasions and as a result of the numerous blood estimations no anæmia developed. The percentage hæmoglobin showed a slight fall during the third month of the diet but was due to iron deficiency since it was corrected by the administration of ferrous sulphate. These observations are in accord with those of other workers in this field [407].

The white cell count averaged 5 000 at the beginning of the experiment

In experimental scurvy the prothrombin time is not appreciably increased [1049]

Capillary Fragility The capillary fragility test as determined by the technique of Gothlin (p 464) remained negative At the end of five months when frank scurvy was present there were fewer petechiæ on the arm after applying a blood pressure cuff for ten minutes at 100 mm mercury than there were in normal controls The negative pressure method of Dalldorf (p 465) also gave negative results This is in keeping with the most recent work on the subject which negatives any connection between ascorbic acid deficiency and increased capillary fragility (p 415)

Blood Pressure This remained constant at 120 systolic 70 diastolic except on one occasion when it dropped temporarily owing to a large loss of blood

Resistance to Infection Contrary to expectations based on the work on ascorbic acid and infection (p 418 *et seq*) there was almost complete freedom from respiratory infection even though the experimental period included the winter months Blood complement determinations were normal throughout the period of observation even when frank scurvy was apparent

Fatigue Fatigue appeared at the beginning of the third month and became progressively more marked Careful tests after prolonged ascorbic acid deficiency showed impaired capacity for aerobic work e g walking and running on the treadmill No great change could be detected in the capacity for doing anaerobic work e g muscular movements measured by an ergograph Tests of harder grade work—a run to exhaustion at seven miles an hour—showed that running was only possible for sixteen seconds whilst following the administration of ascorbic acid the time was increased to sixty six seconds and to eighty four seconds after normal diet had been resumed The performance whilst in the scorbutic state was equivalent to that of a man in the eighth decade of life

Crandon has drawn attention to the fact that scurvy might have appeared much sooner if he had been submitted to extreme muscular fatigue After it had been decided to terminate the experiment fatigue and languor were considerably diminished within twenty four hours of administering ascorbic acid

Farmer [864] has also studied fatigue phenomena in young volunteers kept for five months on a scorbutic diet (0 to 10 mg ascorbic acid daily) A measurable decrease in work output occurred and the subjects complained of severe fatigue after three months on the diet Errors were increased and there was loss of interest in work and motivation Threshold of perception co ordination of motion on a pursuit meter and critical fusion frequency of visual flicker showed little or no change from the normal

Blood Ascorbic Acid The plasma ascorbic acid rapidly fell and reached zero after forty one days on the diet remaining at this level for thirteen weeks before the first evidence of clinical scurvy appeared On the other hand the ascorbic acid level in the white cell platelet layer of the centrifuged blood fell gradually from a relatively normal level of 28 mg per 100 c c on the seventieth day to 4 mg on the eighty second day but did not reach zero until shortly before the appearance of clinical scurvy These observations and the fact that normal wound healing occurred after three months of ascorbic acid deficiency when the plasma level had been zero for forty four days suggest that plasma values are a poor index of the ascorbic acid status of the individual As pointed out later (p 470) the white cell platelet level of ascorbic acid in the centrifuged blood is a more accurate measure of the degree of ascorbic acid deficiency than plasma determination

Miscellaneous Observations The basal metabolic rate fell at one time as low as minus twenty two per cent This was probably due to loss of weight or inanition or both rather than to ascorbic acid deficiency Insulin and glucose tolerance tests were normal Gastric analysis revealed a large drop

in the free and total acid this was restored after administration of ascorbic acid There was a lowering of the total phosphorus content of muscle and an increase of the phosphagen phosphorus All other tests on blood urine the stools and X ray films and electrocardiograms were within normal limits

The opinion is expressed that the long interval that elapsed before clinical scurvy appeared was probably due to the absence of complicating factors such as growth infection or multiple avitaminosis The study proves that clinical scurvy as ordinarily met with is undoubtedly a multiple deficiency disease whereas Crandon was suffering from severe uncomplicated ascorbic acid deficiency It has been observed elsewhere (pp 212 352) that simple vitamin deficiencies do not exist except experimentally

Fox [344] and his colleagues studied nearly a thousand mine labourers in Africa on diets low in ascorbic acid—generally about 15 mg a day Some of their findings can be correlated with those of Crandon For example no changes in capillary fragility were detected there were no signs of anæmia the gums remained healthy resistance to infection was not diminished the healing of wounds was not impaired unless ascorbic acid was *totally* withheld for six months Even when the blood ascorbic acid was nil there was no sign of scurvy Fox and his co workers concluded that deprivation of ascorbic acid unless extremely severe and prolonged does not of itself lead to the appearance of scurvy in the absence of precipitating factors such as infection

Infantile Scurvy This is still seen in children in spite of general improvements in infant feeding Under the names of *Barlow s disease* and *scurvy rickets* it was frequently observed a few years ago Scurvy rickets is a misnomer as a separate entity scurvy and rickets may occur in the same child For the reasons previously given (p 444) infantile scurvy is more prevalent in the artificially fed infant than in the breast fed particularly if the infant is given overboiled dried or condensed milk The disease usually makes its appearance in children of two to twelve months and is rare after eighteen months The greatest incidence is between the seventh and ninth month A case of scurvy at nineteen days has been reported by Follis [428] who regarded it as congenital Faulkner [835] states that the incidence of scurvy among children admitted to the Boston City Hospital is 0 14 per cent Follis [428] observed an incidence of scurvy of 5 3 per cent in 1 303 children autopsied at Johns Hopkins Hospital Baltimore In only nine per cent of the cases was scurvy diagnosed on clinical grounds the remainder were diagnosed radiologically Evans [426] gives a much lower incidence ninety three cases in 106 800 infants or 0 09 per cent diagnosed clinically and radiologically Barlow s observation that scurvy is seldom seen in breast fed infants has been confirmed [428]

The onset of the disease is gradual and the child is usually brought to the family doctor because he screams when his limbs are touched because he does not use his limbs or because of bruising of the orbit and limbs and hæmorrhage from the gums bowel or urinary tract On examination the position

necessary

On examination the infant is usually wasted pale and fretful and is terrified of being touched There may be bruising on the face or body (Fig 1C9) and on deep palpation acutely tender swellings may be felt deep to the muscles particularly of the leg There is a failure to gain weight—which may be masked by œdema—weakness rapid shallow breathing a rapid pulse diarrhœa and vomiting If there is retrobulbar hæmorrhage the eye may be proptosed The temperature is usually slightly raised about 100° F the fever in both clinical and experimental scurvy has never been satisfactorily

INFANTILE SCURVY

FIG 166 Infantile Scurvy I ... Hematoxylin and Eosin ... many between the prede... the pulp at the site of a former hæmorrhage

FIG 167 Infantile Scurvy Photomicrograph of a Premolar Tooth (× 390) Hematoxylin and Eosin The odontoblast layer has been r... fro... the predentine by a previous hæmorrhage Calcification is beginning at the internal surface of the predentine There is a layer of ... which is not collagen on the outer side of the odontoblasts ... have lost their normal morphology

INFANTILE SCURVY

FIG 168 Infantile Scurvy Swollen spongy and hæmorrhagic gums in a child of ten months suffering from scurvy

FIG 169 Infantile Scurvy The same child as in Fig 168 [illegible]

explained, but is probably due to the absorption of blood from the hæmorrhages. Hæmorrhage from the gums (Fig 168) bowel, or urinary tract may be observed but the mouth lesions do not *occur if the infant* is edentulous (cf Adult Scurvy, p 450) The gums are swollen, red and dusky if the teeth are due to erupt. Intracranial hæmorrhage has been reported. A most

INFANTILE SCURVY

FIG 170 Infantile Scurvy Radiogram Note decalcification of the epiphyses with a well defined periphery calcified subperiosteal hæmorrhages which show as a dark shadow on the medial side of the tibiæ and between the tibia and fibula of the left leg, and the dense metaphysial borders of the diaphyses

helpful physical sign is the characteristic ridge enlargement of the costochondral junction [428]

Moderate secondary anæmia is often present with hæmoglobin levels in severe cases as low as forty per cent of normal. Examination of the blood and urine shows the absence of ascorbic acid or markedly reduced levels. A few red blood cells in the urine are of diagnostic importance.

These signs and symptoms are those of frank scurvy. Mild scurvy or scurvy in its early stages can only be diagnosed radiologically (Fig 170). As

a result of the observations of Pelkan [500], Fraenkel [509], Baetjer [499], Wimberger [502], Schwartz [505] and Bromer [500] ten X-ray signs have been recognized :

(1) A zone of rarefaction immediately posterior to the zone of preparatory calcification ; the "scurvy line," the framework marrow (Bromer), the *Gerustmark* of the German writers

(2) A broad, finely irregular edge of dense shadow around the centre of ossification, with rarefaction of the central portion (Wimberger's sign).

(3) A finely irregular broadened intensely calcified zone of preparatory calcification at the epiphyseal end of the long bones, the so-called "white line of Fraenkel."

(4) A small spur at the lateral edge of the epiphysis (Pelkan)

(5) [illegible] appearance

[illegible] g or obliteration

(7) Thinning of the cortical shadow, often represented by a narrow white line

(8) Subperiosteal hæmorrhages, occurring only in the late stage (Fig. 170)

(9) Subperiosteal fractures in the ends of the diaphysis

(10) Enlargement of angulation of the costochondral and vertebral junctions of the ribs

Taken alone the majority of these signs are not pathognomonic Signs (2), (3) and (4) are not absolutely characteristic of scurvy and may also be seen in rickets, lead and phosphorus poisoning and after administering vitamin D preparations The most important sign is (1)

There are three signs diagnostic of latent [illegible]
They are (*a*) a broadened epiphyseal line [illegible]
centre of ossification of the epiphysis , (*c*) [illegible]
shaft Park [514], on the other hand, state [illegible]
logical signs, and that reliance must be placed on early clinical signs. This is disputed by Follis [428], who could only diagnose six out of sixty-nine cases of infantile scurvy clinically.

The healing of scorbutic bone can be demonstrated radiologically after two weeks' treatment [500] At first the broadened epiphyseal line loses its finely irregular appearance and becomes sharply outlined The scurvy line not only disappears but becomes more heavily calcified than the rest of the shaft Years after clinical cure radiological examination still shows oval, definitely circumscribed areas of rarefaction in the interior of the epiphyseal centres of ossification [1000]

Morbid Anatomy and Pathology of Scurvy. The principal lesions are hæmorrhagic and skeletal The former have already been described The skeletal lesions resemble those of the guinea pig, the commonest sites being at the costochondral junctions, the distal ends of the femora, and the proximal ends of the tibiæ and femora and the wrists The lesion is characterized by rarefaction of the cortex and conical widening of the bone, inhibition of bone growth and replacement of the normal junction of bone and cartilage by a zone of connective tissue poor in collagen and containing fragments of densely calcified cartilage with no osteoid tissue These changes result from the inability of the osteoblasts to form normal osteoid tissue in the absence of adequate ascorbic acid with consequent attempts at fibrous tissue union between the epiphysis and diaphysis Epiphyses may be separated and fractures occur

The dental lesions in human scurvy include hyperæmia and œdema of the pulp, degeneration of the odontoblast layer with cyst formation, destruction and calcification of vessels and necrosis and calcification of areas in the pulp. The dentin becomes porotic and resorption occurs along Tomes' canals which widen into spindle shaped and round spaces Abnormal and irregularly canalized dentin is formed The commonest lesions are in the apical third of

the tooth Lesions may occur in the teeth before they do in bone The loosening and falling out of the teeth in advanced scurvy is due to the rarefaction of the alveolar bone

The gingival lesions consist of hyperplasia of the papillæ development of granulation tissue and finally gangrene they do not occur unless teeth are present

In the older literature on scurvy œdema particularly around the ankles (see Fig 161) enlargement of the heart hydropericardium and hydrothorax were described Neuritic changes such as degeneration of the peripheral nerves and large anterior horn cells have been described as well as sensory disturbances and reduced or absent knee jerks Ulceration of the gastro intestinal tract atrophy of various organs and endocrine glands are also mentioned It is almost certain that all these changes do not result from ascorbic acid deficiency, inanition repeated hæmorrhage multiple vitamin deficiencies and infection all play a part

The morphological changes of scurvy are greatly modified by growth activity stress trauma and the presence c
the ascorbic acid requirements Generally
of the lesions diminish with age particularl
affected being those in which growth happens to be most active at the time of the deficiency Stress (motion pressure) exerts an important effect on the ite and extent of hæmorrhage Infections may precipitate scurvy in a person bordering on this condition

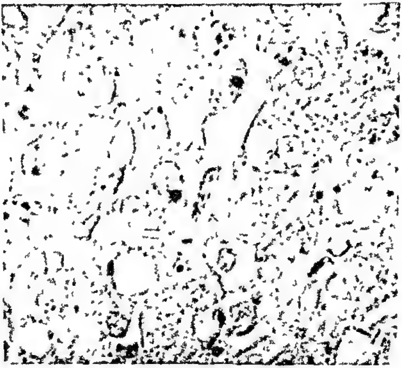
In the scorbutic guinea pig pathological changes occur in the cells of the liver and kidney (Figs 171 173) as evidenced by the deposition of trypan blue in these cells after subcutaneous injection of this dye [1001] Fatty metamorphosis also occurs in the liver cells [472] Aschoff and Koch [39] described advanced fatty degeneration in the liver cells in human cases of curvy although this may have been due to multiple deficiencies Beyer [952] bserved that fatty degeneration of the liver produced by toxins occurred nore readily in scorbutic guinea pigs than in those receiving adequate scorbic acid (Figs 148–150)

Deranged tyrosine metabolism occurs in human scurvy This is discussed n p 427

A close relationship between ascorbic acid metabolism and adrenal ortical function is suggested by evidence from animal experimentation
might therefore be expected
because the blood eosinophil
al and blood glutathione
otassium and sodium in scurvy are normal and unaltered by ascorbic acid herapy [303]

Diagnosis of Scurvy Clinical diagnosis of mild scurvy is difficult and ncertain In frank scurvy in the adult the diagnosis is made on the multiple etechiæ hæmorrhagic manifestations mouth lesions extreme fatigue and ad dietary history In isolated cases the disease may be confused with urpura Mercurial stomatitis may resemble the oral lesions of scurvy but t does not present the other features Acute leukæmia should be considered n the differential diagnosis here the blood count is diagnostic

Diagnosis in the infant is not always easy although the typical drawn p and immobile appearance of the legs (p 169) and the acute pain on ouching the limbs are characteristic of the severe case The finding of a few lood cells in the urine is a most valuable sign Osteomyelitis with its painful mbs and poliomyelitis with its immobility may cause some confusion lthough high fever is against the diagnosis of infantile scurvy and in the atter there is no true paralysis or neurological disturbance Rheumatic ever which is however rare below the age of two syphilitic epiphysitis and Parrot s disease (syphilitic pseudoparesis) may have to be considered Radiological examination of the bones will help to establish the diagnosis



Fig

deficiency

Figs

Special Tests for Scurvy A number of tests for the detection of ascorbic acid deficiency have been devised These are described later (p 464)

Treatment of Scurvy Treatment in uncomplicated cases consists in the administration of fruit juices and large doses of ascorbic acid, e g 200 to 300 mg two or three times a day by mouth taken preferably before or with meals since gastric acid probably assists in absorption There is no need to give ascorbic acid parenterally unless there is nausea or vomiting or the patient has gastro intestinal lesions likely to interfere with absorption The parenteral dosage is somewhat smaller than the oral dose Overdosage is wasteful but does no harm [847] In cases of mild or latent scurvy and infantile scurvy 100 mg daily by mouth is probably adequate Fruit juices are also recommended, as cases have been described that are resistant to pure ascorbic acid Intensive ascorbic acid treatment is continued until the vitamin is freely excreted in the urine or until the lesions are healed and then a maintenance dose of 50 to 100 mg daily given orally

Many cases of scurvy are complicated by other vitamin deficiencies, and these should be searched for and treated An enriched nutritious diet should be given to the scorbutic patient as well as specific vitamin therapy The local lesions are treated symptomatically, e g mouth washes and mild disinfectants for the mouth to prevent infection , light splints for the legs in infantile scurvy

Prevention is better than cure, particularly in the case of infantile scurvy This need never occur if infants, particularly the bottle fed are given fruit and vegetable juices sufficient to supply 20 to 40 mg of vitamin a day (p 445)

SUBCLINICAL SCURVY, THE PRESCORBUTIC STATE, SUBVITAMINOSIS C, AVITAMINOSIS C (HYPOVITAMINOSIS C, PARAVITAMINOSIS C, ASYMPTOMATIC SCURVY)

A state of ascorbic acid deficiency without the clinical manifestations of scurvy has been described under the above names Diagnosis is not made on definite clinical grounds, but usually from laboratory tests or from the dietary history The conception of subclinical forms of scurvy is due to Hess [447] who as far back as 1917 pointed out that an asymptomatic stage of scurvy with well marked skeletal lesions may precede clinical infantile scurvy It has been observed that skeletal lesions characteristic of scurvy can be demonstrated in animals suffering from ascorbic acid deficiency without clinical signs of scurvy [449] In the human being a group of symptoms characterized by susceptibility to infection, unreasonable loss of vitality, lessened endurance, gingivitis, vague pains, bodily fatigue, loss of appetite and mild digestive disturbances have been attributed probably incorrectly, to ascorbic acid deficiency Certainly a low degree of excretion has been detected in persons showing such a group of symptoms but, on the other hand it is known that an individual may excrete very little ascorbic acid and have low plasma values and yet be in excellent health Dahlberg Engel and Rydin [836] carried out carefully controlled observations on five thousand soldiers and concluded that half of them suffered from ascorbic acid deficiency as judged by urine and saturation tests yet their health was as good as the other half who received an extra 50 mg of ascorbic acid daily Many workers state that they have been unable to recognize " subvitaminosis C as a clinical entity and deprecate the use of this expression applied to subjects unsaturated with ascorbic acid or who excrete only small amounts Hjarne [911] has been unable to correlate the general clinical condition of children with their blood ascorbic acid level

On the other hand children in the poorer classes sometimes receive suboptimal amounts of ascorbic acid [837 839] and respond to improved diets [451] The beneficial effects of the so called Oslo breakfast with its fresh vegetables or salad, has been observed by school medical officers The

results may be due to improved nutrition generally and not to an increased intake of ascorbic acid only It has been stated with very little evidence
fatigue in the spring [451]
ires of ascorbic acid over

Many cases of inflammatory dental disease such as gingivitis have been attributed—probably incorrectly—to lack of ascorbic acid and not so much to lack of dental hygiene [995] It is unlikely that the average case of
e p 476)
prevalent in states of ascorbic
icile with the observations on
scurvy and anæmia (pp 453 454) and with the observation of Lozner [457] that normal regeneration of hæmoglobin can occur in patients whose plasma contains no ascorbic acid

So called ascorbic acid deficiency as judged by urine saturation and blood tests has been detected in a number of patients suffering from various clinical conditions and there has been a tendency to associate these ætiologically with ascorbic acid deficiency This is an uncritical approach because apparent ascorbic acid deficiency judged by laboratory tests can be demonstrated in apparently healthy controls It is far more likely that in the case of the diseased patient his disease produces a conditioned ascorbic acid deficiency Thus infections and diseases with increased metabolism may increase the requirements of ascorb
its absorption and the p
in nervous and mental
hence of ascorbic acid The remarks on conditioned vitamin deficiency in the chapter on Aneurine (p 223) apply equally well to ascorbic acid Croft and Snorf [521] estimated the blood ascorbic acid of a hundred unselected patients and of these thirty eight were considered to be suffering from ascorbic acid deficiency as their blood levels were below 0 4 mg per 100 c c Most of them suffered from gastro intestinal conditions which undoubtedly conditioned the deficiency None however showed any definite scorbutic signs

Gingival Manifestations Kruse [995] states that macro and microscopic examinat
convenient and objective
method c
any cases he says that the
gingival
e in some only by bio
microscopy Both gums are not equally affected the upper gum often although not invariably showing the more advanced process The sites affected are involved in a definite order interdental papillæ the marginal gingivæ and then the alveolar gingivæ Kruse divides the changes into acute and chronic with three stages in each

In the *acute process* the subsurface vascular papillæ are engorged and dilated and under the microscope enlarged and congested capillaries are visible In mild cases this change is restricted to the interdental papillæ and then to the marginal gingivæ In more severe cases the vascular reaction may be seen all over the gum At this stage there is no swelling In the second stage the gum is reddened first at the points of the interdental papillæ spreading to the bases and then the marginal gingivæ In more marked cases the
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reddened gums swell and although the changes may be restricted to the interdental papillæ more often the marginal gingivæ are affected and form a red swollen collar projecting around the necks of the teeth Frequently the entire gum is red and swollen and the swelling may be so intense as to stretch the gum and give it a glossy appearance The gum may recede slightly from its free edge increasing the length of the crown and if it is very swollen a

sulcus may form between the *gum and teeth* becoming filled with calculus or infected *debris* Infection of the gum and bleeding gums are common m

ch the gums are congested
h necrotic material Later
process becomes necrosed

In the chronic process the subsurface vascular papillæ show slight dilatation and engorgement so that gums appear reddened and slightly swollen the process commencing in the interdental papillæ extending to the gingival margin and finally over the whole gum In the second stage the redness and *swelling are slowly masked by* œdema and infiltration so that the gum appears swollen and pale The process may be confined to the papillæ or spread over the whole *gum* Atrophy *in the form of pitting* occurs in the third stage The pits or depressions occur first on the interdental papillæ then on the gingival margins and finally over the whole gum Although often visible macroscopically the pits are best seen microscopically In the next stage the atrophy becomes more profound and the pits gradually disappear The papillæ get smaller and recede from the gum leaving the cement exposed Finally the whole gum shows pronounced atrophy becomes white in colour and the teeth are loosened and extruded

Kruse states that these changes are reversible by giving ascorbic acid He remarks that in all cases a long period of time is needed for recovery more than a year in some cases even with intensive treatment

Kruse s *observations conflict with those of Crandon [68] Farmer [1078]* and the Medical Research Council team [407] After six months on a scorbutic diet Crandon failed to observe any pathological changes in his teeth and gums although the level of ascorbic acid in the plasma and white cell layer was z

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workers stated that gum changes only appear after the skin lesions As in Crandon s case the level of ascorbic acid in the plasma and white cell layer was zero Kruse s work has been severely criticized by King [536] who states *(His) conclusions* disclosed such unfamiliarity with gingival pathology and gingival capillaroscopy as to render them of dubious value

Gums in apparently normal people often bleed on touching with a probe Redness swelling and bleeding *of the gums can be caused by* trauma and œstrogens and can occur during menstruation and pregnancy and as a result of over smoking

LABORATORY METHODS USED FOR DETECTING ASCORBIC ACID *DEFICIENCY*

Capillary Fragility Tests Tests involving the measurement of capillary *fragility were the first to be used for the detection of ascorbic acid deficiency* In the *positive pressure method* devised by Gothlin [82 83] also known as the Rumpel Leede test the exact technique is as follows A circular area 60 mm in diameter is marked *off in the antecubital fossa* and a blood pressure cuff placed at least 2 5 cm above this and pumped up to 50 mm mercury pressure which is maintained for fifteen minutes After the pressure is reduced the number of petechiæ is counted *If many are present the test is repeated* not less than forty minutes later on the other arm at a lower pressure of 35 mm of mercury The results are graded according to the following scheme —

- Grade I No petechiæ within the examined skin area at 50 mm pressure for fifteen minutes
- Grade II Petechiæ appearing at *50 mm mercury* but less than six in number

Grade III More than six petechiae at 50 mm mercury but two or less at 35 mm mercury

Grade IV At least two petechiae at a pressure of 35 mm mercury

The pressure of the cuff must be infradiastolic so that there is an uninterrupted flow of blood to the forearm.

The test is carried out at room temperature (16°–21° C) and the patient should not have a hot bath on the day of the test or take any exercise within three hours of it. Gothlin considers that more than six petechiae at 50 mm pressure for fifteen minutes are abnormal and he believes that this capillary fragility test serves as a measure of the physiologically indispensable minimum requirement for ascorbic acid. From simultaneous ascorbic acid tests on blood a positive Gothlin test is stated to correspond to a blood level of between 0.1 and 0.14 mg of ascorbic acid per 100 c.c.

Wright [522] modified the test by inflating the pressure cuff midway between the systolic and diastolic pressures for fifteen minutes and then counting the petechiae in a 2.5 cm circle on the flexor surface of the forearm. A petechiae count of 0 to 10 is considered normal, from 10 to 12 borderline and above 12 abnormal.

Dalldorf [523] employed a *negative pressure method* for determining capillary fragility. He applied a 1 cm suction cup to the skin of the upper arm near the deltoid insertion and applied varying pressures for one minute. If petechiae appeared at a negative pressure of 25 mm of mercury or below, the capillary fragility was considered abnormal.

Scarborough [510] determined the capillary resistance in three standard areas on the arm using negative pressure applied for half a minute.

The capillary fragility test has been widely used but the results obtained are not consistent. Re-investigation of the test by a number of workers has shown that it is of no value in the diagnosis of scurvy or ascorbic acid deficiency [66, 68, 81, 347, 350, 526, 532, 553, 1010]. For a more detailed discussion see p. 415.

The Intradermal Test. In 1937 Rotter [533] described a test for the diagnosis of ascorbic acid deficiency depending upon the power of the ascorbic acid of the skin to decolorize the blue dye 2:6 dichlorophenolindophenol. His test consisted of injecting 0.1 ml of a 1 in 400 dilution of the dye into the skin of the volar surface of the forearm. According to Rotter if the individual is saturated the ascorbic acid in the skin decolorizes the dye in less than five minutes; normal cases decolorize it in five to ten minutes and unsaturated individuals require longer than ten minutes. Shortly after its introduction several workers used the test and considered it adequate as a rough test to estimate ascorbic acid deficiency [534, 535, 1072] and Slobody and his co-workers [1002] who modified the test considered that there was a correlation between Rotter's decolorization time and the plasma ascorbic acid level. They injected 0.05 ml of N/300 dye solution and stated that decolorization took more than fourteen minutes if the plasma ascorbic acid was below 0.3 mg per 100 ml.

More recently a number of other workers have submitted the test to extensive trial but they have found no correlation between the decolorization time of Rotter and the clinical condition of the patient or between it and ascorbic acid levels in blood or urine [537, 541]. Technically it is difficult to inject small quantities of solution with precision but even using an improved syringe capable of injecting exactly 0.01 c.c. Goldsmith [540] could obtain no correlation between the intradermal test and blood serum values. Changes in position and temperature considerably affected decolorization time. Goldsmith subjected the test to a statistical study and her results indicated no specificity for the method. MacLenathen [539] found that the variations in the decolorization time were too great for any normal standard to be set up and there was frequently a wide variation in the response at

areas within a few centimetres of one another Holland [488] found that variations in decolorization time occurred when the test was performed simultaneously on both arms Other factors not considered in devising the test are the possibilities of other reducing substances in the skin and local changes in the circulation oxygen supply, and lymphatic drainage of the forearm With all these possible variables the test does not appear to have any clinical value

Urinary Excretion of Ascorbic Acid Single determinations of ascorbic acid in the urine are of no value as an index of ascorbic acid nutrition The twenty four hour excretion has been used but this is equally valueless as it only reflects the immediate intake and not the extent to which tissues are affected Twenty four hour collections of urine are preserved by adding enough twelve per cent metaphosphoric acid to the receptacle to maintain a final concentration of two to three per cent or two per cent oxalic acid is added An alternative but less satisfactory method is to add 10 ml of glacial acetic acid to every 100 ml of urine, which is kept in a refrigerator or dark bottle 8 Hydroxyquinoline is also a good preservative Using acetic acid there is a loss of ten per cent of ascorbic acid for every hour s storage with phosphoric acid the loss in the same time is only one per cent [1003] Wright [555] has shown that even with preservative a total loss of twelve per cent of the ascorbic acid may result on storing the urine until the next day , in warm weather with partially filled bottles the loss may reach forty six per cent [556] The principle of the estimation is to take a measured amount of recently standardized 2 6 dichlorophenolindophenol and determine the amount of urine which has to be added to it to discharge its colour the titration being carried out rapidly The dye is made up in aqueous solution at a strength of about 0 1 per cent so that 0 05 ml (i e 0 5 mg) is equivalent to roughly 0 025 mg of ascorbic acid The solution should be freshly prepared and standardized against crystalline ascorbic acid 0 025 gram of which are dissolved in 50 ml of water for the purpose of standardization The purity of the ascorbic acid is further checked against 0 01 N *iodine solution* In titrating the urine a 2 ml microburette reading to the nearest 0 01 ml is used The urine is run from the burette into the dye solution conveniently contained in a pointed centrifuge tube

2 6 Dichlorophenolindophenol is also decolorized by other substances present in the urine, e g drugs [559] and some normal products of metabolism (p 392) Richter and Croft [1003] estimate the true ascorbic acid in urine by eliminating thiosulphates and sulphur containing substances in the urine with lead acetate before titration with indophenol dye Another method of estimation using indophenol dye is to add excess of the latter and extract the excess with xylene the difference giving the amount reacting with the ascorbic acid in the urine [459] The use of hydrogen peroxide and formaldehyde eliminates the interference of iron sulphites and reductones [459]

Ascorbic acid can be estimated in urine by an entirely specific method depending on

crystalline 2

Excretion

The excretion in health and disease varies enormously from almost zero to 50 mg daily (p 437) Kassan and Roe [367] examined a series of apparently normal medical students and found that fourteen excreted no ascorbic acid yet they had no signs or symptoms of ill health Roe and Hall [360] state that no less than fourteen out of fifty subjects on an apparently adequate diet showed no excretion of ascorbic acid Pijoan [873] lived on a diet poor in ascorbic acid and excreted none in the urine for twenty months without any ill effect Fox [344] has repeatedly observed low excretions for African natives in good health Conversely the urinary excretion of ascorbic acid is not always as low as would be expected in cases of frank avitaminosis Schultzer [369] for example found mean daily excretions in scorbutic

patients ranging from 13 to 24 mg According to some observers a daily excretion of 24 mg of ascorbic acid would indicate an adequate intake of the vitamin Kellie and Zilva [359] have shown that the amount excreted can be made to vary at will by adjustments of the diet

There are several factors including a fluctuating renal threshold which may affect the urinary excretion of ascorbic acid besides a diminished intake (p 437) Deeny [970] has shown that marked hourly variations occur in the excretion on a standard intake and at all physiological levels of intake The position is well commented on by Sinclair [453] There is no reason why a person in full health should be expected to excrete any ascorbic acid , he is not expected to excrete glucose in the urine

Saturation Tests (Test Dose) The urinary excretion of ascorbic acid following the administration of a test dose of the vitamin has been used as a criterion of the body stores and as a means of detecting early states of ascorbic acid deficiency The rationale of the test is based on the hypothesis that following the administration of ascorbic acid the requirements of the tissues for the vitamin are satisfied before its concentration in the blood rises to the threshold value and is eliminated in the urine If a test dose of ascorbic acid is given to a normal subject receiving 25 to 50 mg daily a large rise in the urinary excretion of ascorbic acid occurs on the first day of the test or certainly on the second if the ascorbic acid intake is lower than normal the response is less or delayed by one or more days [560 866]

Oral Test Dose In the original method of Harris and Ray [385] twenty four hour specimens of urine were collected on consecutive days to determine the resting level of excretion and response after repeated daily test doses noted Then Harris and Abbasy [356] introduced a simplified method When first seen the subject emptied the bladder three hours later a specimen of urine was obtained any ... and the whole titrated for ascorbic acid ... on the second and third day The ne ... orbic acid per 10 stones of body weight was given in water and the urine collected three to six hours later i e the time of peak excretion The test was repeated on subsequent days until a sharp rise in excretion occurred According to Harris subjects on an intake of 25 to 50 mg of ascorbic acid per 10 stones body weight showed a sharp rise in the urinary excretion of ascorbic acid on the first or certainly the second day of test dosing Harris calculated what he considered to be the body deficiency of ascorbic acid by multiplying the test dose by the number of days dosing needed to obtain a sharp rise in the urinary excretion of ascorbic acid In a later paper Harris [1004] defines saturation as sufficient storage of ascorbic acid in the body that an excretion of 50 mg or more occurs within four to five hours of a test dose of 700 mg ascorbic acid per 10 stones body weight With an intake of 45 to 75 mg or more of ascorbic acid daily a response occurs on the first day of test dosing On an intake of 37 mg ascorbic acid daily the response occurred on the first or second day of dosing on 23 mg daily the response is on the second or third day Some of Harris s test subjects required ten days dosing for saturation

Since the original work of Harris in 1935 many workers have devised methods for assessing the level of ascorbic acid nutrition by a test dose technique The test doses vary from 200 to 600 mg Beck and Schorlemmer [568] and Gander and Niederberger [569] collect the urine over a period of six hours after a 300 mg test dose and consider that a concentration of 5 mg or more of ascorbic acid per 100 ml urine is adequate Pemberton [562] gives a test do ... in the excretic ... found that it ... Engelfried [90 ... a test dose of ... mg is satisfactory These methods have been criticized by Richardson and

Mayfield [561] who question the validity of the six hour period They find that the volumes of urine and the amount excreted at each urination differ considerably even in the same person and they could find no correlation with the excretion at six hours and twenty four hours

Others have given a test dose of 200 to 600 mg ascorbic acid and consider an excretion of fifty per cent or more of this within twenty four hours or twenty five per cent in twelve hours a satisfactory index [376 462 563 877] Spellberg and Keeton [337] and Youmans [565] consider an excretion of thirty per cent of a test dose in twenty four hours an adequate index of satisfactory intake Wright [566] regards an excretion of less than twenty per cent in twenty four hours a sign of suboptimal intake Baumann [564] gives a daily dose of 50 to 100 mg of ascorbic acid daily and states that normal subjects excrete sixty to eighty per cent of the test dose on the third or fourth day

Parenteral Test Dose Some workers give the test dose parenterally This route is unnecessary because it has been shown that ninety nine per cent of a test dose given orally is absorbed (p 434) It may also give erroneous results because if given intravenously or even intramuscularly ascorbic acid quickly reaches the blood stream and begins to be excreted before the tissues can absorb it The size of the test dose and the interpretation of the results are even more variable than with the oral test dose Berryman and others [905] have shown that there is a considerable variation in the amount excreted after the intravenous injection of 200 mg ascorbic acid even on a fixed oral intake of 100 mg in the diet Wright [570] gives the very large dose of 1 000 mg of ascorbic acid intravenously and considers an excretion of 400 mg after five hours or 500 mg after twenty four hours an index of adequate intake Ralli and her co workers [572] regard an excretion of 40 mg within three hours after an intravenous test dose of 100 mg as evidence of saturation Kastlin and Schlesinger [573] found that normal subjects excreted forty per cent of an intravenous dose of 500 mg within four hours Van Eekelen and Heinemann [574] warn against the erroneous conclusions that might be drawn from the intravenous injection of large doses and employ a dose of 4 mg per kilo subcutaneously Practically the whole of this is excreted in six hours peak excretion occurring at three hours Remel and Schenk [611] consider an excretion of less than 18 to 20 mg of ascorbic acid after an injection of 200 mg indicates a deficient intake

Several investigators [240 550 576] have pointed out that in saturation tests a delay in excretion may occur in persons with impaired renal function (see p 438) Ludden and Wright [550] state that erroneous values may be observed in such persons when the ascorbic acid content of single urine specimens obtained three five six or eight hours after any test dose of whatever size are used as criteria of the state of ascorbic acid nutrition They have introduced a modified technique which they say is valid even in cases of impaired renal function with the exception of uræmia The test is conducted as follows

The patient omits breakfast on the morning of the test Immediately after he has micturated and discarded the preliminary specimen of urine ml of normal saline is given intravenously A exactly one and a half hours after the injection rs after The twenty four hour output can then be predicted from the formula $\dfrac{ab}{1\ 26\,a - 0\ 27\,b}$ where a and b are the ascorbic acid excretions over one and a half and five hours respectively Wright and Ludden term this expression the *Saturation index* The formula is arrived at from a definite correlation between the percentage of the first five hours excretion of ascorbic acid excreted during the first hour and a half and the percentage of the twenty four hours excretion excreted during the first five hours

Criticism of Test Dose Studies In criticism of the test dose technique for detecting ascorbic acid deficiency it must be admitted that it has not yet been demonstrated that 'saturation with ascorbic acid is a normal state, in fact it is definitely abnormal because very few subjects are saturated with respect to ascorbic acid if they rely on natural sources of the vitamin Further many people in the best of health have been shown to be unsaturated As Crandon [68] has shown it is possible to feel in good health and excrete no ascorbic acid *There is no reason why ascorbic acid should be excreted in the urine* any more than glucose Excretion and saturation tests do not measure the ascorbic acid reserves of the white cell layer and the tissues, which retain their ascorbic acid even when the urinary excretion is low

The measurement of the level of ascorbic acid nutrition by the test dose technique is a purely arbitrary procedure A massive and unphysiological dose of ascorbic acid is given and the excretion studied over a period of several hours the dose varying from one worker to another The interpretation of the results also depends upon the individual worker Some give daily supplements until there is a sudden rise in the urinary excretion others consider that the subject is saturated when fifty per cent of a test dose is excreted If these arbitrary figures are accepted and there is no reason why they should be there are still sources of error The renal threshold is variable (p 437) the excretion of ascorbic acid also depends upon the acid base equilibrium and the composition of the diet and Deeny [970] has shown that

varies markedly from hour to

alue of the test dose technique

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hours after giving the test dose Berryman and his co workers [905] have shown that there is considerable variation in the ascorbic acid excretion after a test dose

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Estimation of Ascorbic Acid in the Blood (The 2 6 dichlorophenolindo phenol titration test has been applied to the estimation of ascorbic acid in blood) A number of techniques using this test have been developed for the estimation of ascorbic acid in the plasma [360 577–586] Oxalated blood is first centrifuged and the plasma deproteinized with metaphosphoric acid After recentrifuging the clear liquid is titrated against the dye indicator or methylene blue as for urine (Using a methylene blue micro method Butler and Cushman [1006] estimate the ascorbic acid in 0 2 ml of capillary blood A method that is commonly used is that of Roe and Kuether [360] which depends on the reaction between 2 4 dinitrophenylhydrazine and dehydroascorbic acid to form a red compound which can be estimated colorimetrically Isolated analyses of blood plasma values have proved quite worthless in estimating degrees of ascorbic acid subnutrition Estimation of the plasma ascorbic acid is only a measure of the immediate nutritive or metabolic level and is dependent on the recent dietary intake It does not reflect the tissue stores)

High fasting values may indicate satisfactory nutrition [466] but low values do not provide a reliable index Healthy persons have ascorbic acid plasma levels ranging from 0 1 mg per 100 ml [591] to 2 48 mg per 100 ml)

values indicated that conclusions o

on plasma levels could be entirely

[1035] found that even after admi

daily for six months the final plasma ascorbic acid was no different from that

of controls The threshold value may vary in different individuals (p. 437), and plasma values are higher during menstruation [1007] Some observers consider that plasma values below 0 5 to 0 7 mg per cent indicate a state of ascorbic acid depletion, and that values from 0 7 to 1 mg indicate mild deficiency These figures are purely arbitrary ones, based on "saturation" tests, and there is no clinical justification for them Ralli [878] and her associates believe "saturation" to be the ideal, with blood plasma values of 1 mg. per 100 ml or over. According to Bryan [466] and his co workers saturation occurs with intakes of 1 7 to 1 9 mg. of vitamin per kilo of body weight It has never been satisfactorily demonstrated that the body is saturated with respect to ascorbic acid in normal healthy persons

On a daily intake of 60 to 80 mg of ascorbic acid, which covers the recommended intake of the National Research Council, U S A, the plasma ascorbic acid is about 0 7 mg. per 100 ml The general opinion is that a fasting plasma level of 0 6 to 0 8 mg. or more is adequate, although this range is purely arbitrary and there is no justification for it.

Crandon [68], Fox [344] Rietschel [342] and others have shown that plasma ascorbic acid may be exceptionally low without clinical evidence of ascorbic acid deficiency. According to Rietschel plasma levels of 0 2 to 0 3 mg are not abnormal He kept a subject on a diet free of ascorbic acid for one hundred days without producing scurvy; the ascorbic acid plasma level was zero Pijoan [873] also maintained a plasma level of 0 0 to 0 2 mg per 100 ml for twenty months without ill effects Dagulf [463] reports that three hundred and twenty six healthy Swedish people had an average plasma ascorbic acid of 0 22 mg per 100 ml In scurvy, too, the values are not always exceptionally low, though they all fall within the range of 0 0 to 0 5 mg There is certainly complete lack of correlation between the clinical condition and plasma ascorbic acid values Thus Croft and Snorf [521] noted blood values of only 0 12 mg, and Kassan and Roe [454] levels of 0 02 mg, without any signs of scurvy Owens [257] has also observed levels below 0 4 mg in fifty diabetics without clinical evidence of ascorbic acid deficiency Holmes [637] examined the plasma ascorbic acid in sixty healthy children and low levels were observed, which persisted over a period of ten months The ability to maintain a fixed level of ascorbic acid in the plasma, no matter how small indicates a positive ascorbic acid balance [873]

(There is evidence that accurate information on the level of ascorbic acid nutrition can be obtained from analyses of whole blood [301, 360, 408, 477, 714] or of the white cells and platelets [588–590] Bessey, Lowry and Brock [413] have devised a method for estimating ascorbic acid in whole blood using 0 1 ml of blood Butler and Cushman [590] have shown that whole blood, and the white layer of the centrifuged blood of subjects whose ascorbic acid nutrition would be considered poor may contain measurable amounts of ascorbic acid (25 to 38 mg per 100 ml) even when the plasma level is zero) Crandon [68], whilst on a scurvy producing diet, noted that after forty one days his plasma ascorbic acid was zero, but even after eighty two days the white cell platelet value was 4 mg per 100 ml (It would appear that ... hole blood or of the white blood cells ... from infection or leukæmia provides ... id deficiency than plasma values

Whole blood determinations afford the best index for "saturation" What is important is the tissue reserves of ascorbic acid (not the plasma level), which is best measured by the concentration in whole blood or the white cell layer) If this falls steadily the intake of ascorbic acid is inadequate According to Butler and Cushman [590] the average normal ascorbic acid of the white cells and platelets is 34 mg per 100 grams, with a range of 29 to 43 mg Even massive doses of 1 gram daily do not raise it above normal levels [1034] They have shown that ascorbic acid passes from the plasma to the red blood cells and that the distribution ratio of the plasma concentration to the red

cell concentration varies with the state of ascorbic acid nutrition. The distribution between plasma and whole blood is related to the level of ascorbic acid in the blood. At whole blood levels below 0.6 mg. per 100 ml. the plasma content is lower than the whole blood content; at whole blood levels above 0.9 mg. per 100 ml. the plasma content is higher than that of whole blood [388, 397]. In patients with leukæmia the concentration of reducing substance in the white cell layer is as high as 140 mg. per 100 ml. when the plasma ascorbic acid is as low as 0.2 mg. If the plasma level of ascorbic acid were taken as an index of ascorbic acid nutrition these patients would have been considered to be suffering from considerable deficiency.

The plasma values do not reflect the amount of ascorbic acid stored in the body; they merely represent an overflow and when this is high enough ascorbic acid is excreted. (Whole blood values indicate the extent of saturation down to a state of marked depletion and while values above 5 mg. per 100 ml. exclude scurvy, values below this are equivocal.) Farmer [864] observed zero plasma ascorbic acid values in volunteers on scorbutic diets without clinical evidence of scurvy. It took seventy days on the diet for the plasma ascorbic acid to fall to zero. (White blood cell concentrations of ascorbic acid are the best indication of ascorbic acid deficiency. Technical difficulties attached to estimating its concentration in white cells have prevented its routine use.)

Blood tests for ascorbic acid deficiency have been used in which a test dose of vitamin is injected intravenously or intramuscularly and the blood levels studied at varying intervals afterwards. Kajdi [592] and her co-workers proposed a new saturation test in which the plasma ascorbic acid is determined before and four hours after the injection of 200 mg. of the vitamin. From these figures is calculated the *ascorbic acid index*, which is defined as the initial plasma value multiplied by the increase in the plasma four hours after the injection of the vitamin multiplied by 100. Kajdi takes a four-hour interval because she finds that the ascorbic acid blood plasma level reaches a constant value by that time. The test dose of 200 mg. was chosen because smaller amounts did not sharply illustrate the difference between depleted and partially depleted stores of ascorbic acid, and larger doses were no more accurate. Kajdi states that an ascorbic acid index below 0.8 indicates clinical scurvy, indices between 0.8 and 6 show a low reserve, and an index of 10 or over corresponds to optimal ascorbic acid nutrition. It is claimed that in sixty-four out of seventy cases correct information on the ascorbic acid status of the patient was obtained, laboratory findings being checked against the condition found on clinical examination. Elmby and With [584] employ a saturation test using 500 mg. of ascorbic acid and determine the plasma level before the injection and eight times within the following four hours. This seems an unnecessary elaboration with much inconvenience to the patient.

Goldsmith and Ellinger [567] examine the plasma ascorbic acid in the fasting patient and again one and three hours after the administration of 600 mg. of ascorbic acid. They consider that the ascorbic acid status is adequate if the original level is over 0.7 mg. per 100 ml., rising to 2 mg. or more three hours after the test dose. Similar tests have been employed using smaller intravenous test doses (100 to 300 mg.) [593, 594, 842].

A method of estimating ascorbic acid deficiency based on a test dose of 500 mg. intravenously with observations on both the blood level and
excretion over a fou
[573]. They claim
to the urinary excretion in a twenty-four hour period. Ascorbic acid is first estimated in a blood sample and specimen of urine after fasting for twelve to fifteen hours, and then 500 mg. of the vitamin given intravenously. A blood specimen from the opposite arm is collected five minutes later. Subsequent blood and urine specimens are obtained one, two, three and four hours later

per cent and the vitamin excreted he total urinary excretion in milli e excreted are computed Kastlin al curve of a saturated person shows the following characteristics (a) The fasting blood level of ascorbic acid is 0 7 mg per cent or higher (b) The five minute period is relatively high—from 4 5 to 9 mg per cent (c) The rate of return of the blood concentration from the five minute peak to the four hour level is gradual showing that the tissues have no great avidity for ascorbic acid (d) The four hour blood level is well above the fasting level (e) Urinary excretion of the test dose is greatest in the first hour and the total urinary excretion in four hours is forty per cent or more of the test dose

The typical severe ascorbic acid deficiency curve is stated to show a marked deviation from the normal (a) The fasting blood level of ascorbic acid is below 0 4 mg per cent (b) There is only a slight variable rise in the blood level after five minutes (c) The blood concentration falls rapidly to near the fasting level (d) The total urinary excretion ranges from a few milligrams to twenty per cent of the test dose The authors of this test state that it may show evidence of severe vitamin deficiency when there are no clinical signs of scurvy

Rinehart and Greenberg [785] read the plasma ascorbic acid three and five hours after an oral dose of 15 mg per kilo of body weight For the sake of simplicity they classify the resulting blood plasma curve as flat (peak below 0 5 mg per cent) medium (peak 0 5 to 0 9 mg per cent) or high (peak above 0 9 mg per cent) According to Rinehart and Greenberg the flat curves reflect tissue depletion of ascorbic acid and the medium curves moderate depletion

These plasma studies like others described are based on arbitrary standards and ignore the fact that blood plasma levels do not reflect tissue stores but only the immediate intake and can be very low in the absence of scurvy or ill health The urinary excretion is an overflow from the plasma

Ascorbic Acid in Cerebrospinal Fluid Rohmer Bezssonoff and Sacrez [1011] state that the level of ascorbic acid nutrition can be followed by estimating the vitamin in cerebrospinal fluid There is no parallelism between the cerebrospinal fluid levels and the urinary excretion Owing to the greater trouble of obtaining cerebrospinal fluid the necessity for the patient to remain in bed after spinal puncture and all the risks attending the removal of cerebrospinal fluid the method has little to recommend it

ASCORBIC ACID IN THERAPEUTICS

The only disease that specifically responds to ascorbic acid is scurvy yet its use has been advocated in the treatment of a number of conditions

With regard to dosage and administration there is no need to give the

In severe cases of scurvy relief may be more rap[illegible]
Intravenous injection is not recommended as the renal threshold may be rapidly exceeded and a large part of the dose excreted in the urine Intramuscular injection is therefore preferable As a dietary supplement 100 mg a day in divided doses is adequate When given therapeutically there is no justification for repeated doses in excess of 100 to 200 mg as once the plasma level begins to rise the excess spills over into the urine (p 437) Farmer [864] has shown that when the plasma and white cell ascorbic acid are zero the administration of 100 mg daily will eventually produce tissue saturation A patient grossly deficient in ascorbic acid requiring it parenterally may need

500 mg daily for several days until the tissues are satisfied It should be taken several times a day rather than in one large dose

Ascorbic Acid in Infections The results of experimental studies on immunity and infection are very conflicting (pp 418–421) In human infections there is certainly a fall in plasma and urinary ascorbic acid but this is almost certainly due to redistribution the vitamin passing from the plasma into the white cells (p 420) and possibly into the adrenals (p 430) Ascorbic acid is bacteriostatic and bacteriocidal but only in concentrations much higher than can ever be achieved in the tissues On the basis of the lowered excretion during infective states many workers have given large doses of ascorbic acid to patients suffering from infections There is no evidence from controlled observations that this procedure has any effect beneficial or otherwise on the course of the infection or the condition of the patient Assuming that ascorbic acid does play a part in immunity phenomena and leucocytosis (p 420) the normal intake is presumably sufficient for this purpose As long as a patient is excreting ascorbic acid in the urine the needs of the body are being satisfied Brown [509] and his co workers state There is no clear cut unchallenged demonstration that ascorbic acid can exert a direct effect against a clinical bacterial infection

Diphtheria The evidence that ascorbic acid has a protective action *in vivo* against diphtheria toxin is conflicting (p 419) Whether the administration of ascorbic acid as a therapeutic measure has any effect on the progress of the disease clinically is very doubtful It is claimed by some workers that the administration of ascorbic acid to patients suffering from the toxic form of diphtheria lowers the mortality rate [604 606] This has been denied by others who have observed no favourable effects [486 607 617] Ascorbic acid has no effect on the Schick reaction [598 608]

Pneumonia Tonutti has shown that in pneumonia large amounts of ascorbic acid are taken up by the white cells (p 420) Gander and Niederberger [432] have attempted to correlate the seasonal incidence of ascorbic acid deficiency with that of pneumonia according to them the peak mortality months for pneumonia coincide with those for the lowest level of intake (about March) The figures of Anderson [931] do not bear this out His mortality figures for pneumonia in Glasgow show that the highest mortality is in January and the quarter preceding it He states that the number of deaths in January is twice the average of the other eleven months According to Glazebrook and Thomson [816] the incidence of pneumonia was lower in a large group of adolescent boys receiving supplements of ascorbic acid than in

Gander [illegible] een patients with ascorbic acid and recorde [illegible] occurred if the patients were saturated with the vitamin on the first day of the illness Then the temperature dropped by cris [illegible]

general condition [illegible]

was described The [illegible]

and 300 mg by mouth [illegible] of ascorbic acid by mouth was given [illegible] No controls were used in this study Hochwald [609 610] states that if 500 mg of ascorbic acid is given intramuscularly every two hours an attack of lobar pneumonia can be cut short on the first day If given on the second day the course of the disease can be shortened [illegible]

of the disease less [illegible]

ment in general co [illegible]

improvement in t [illegible]

Kalk and Frobenius [613] and Wuther [614] consider that pneumonia is an allergic hyperergic condition a nasopharyngeal infection precedes the lobar pneumonia by two or three weeks and sensitizes the body Others have claimed that large doses of ascorbic acid produce clinical improvement [612, 615 616]

Tuberculosis. A diminished excretion of ascorbic acid and low plasma levels have been reported in tuberculous patients [126, 127, 633, 634, 849, 850]. As in other infections, these low levels are due to redistribution of ascorbic acid in the body. As healing in tuberculosis is characterized largely by the formation of connective tissue, for which ascorbic acid is essential, it is possible that ascorbic acid deficiency may delay healing and have an unfavourable effect on the course of the disease. Getz and Koerner [849] state that an extreme degree of ascorbic acid deficiency appears to make the prognosis worse in tuberculous patients. In a follow-up paper based on a study of 1,100 men Getz and his co-workers [1030] concluded that marked vitamin A and ascorbic acid deficiency occurred in the 28 men of this group who developed pulmonary tuberculosis. Other factors, e.g. poor housing and living conditions, exposure to infection, protein intake and occupation, which were not taken into account, may have been contributory. Other workers have failed to observe any parallelism between the severity of the disease and plasma and urine levels [493, 633].

Animal experiments suggest that ascorbic acid deficiency predisposes to tuberculous infection. Thus Greene [619] and his co-workers found a shortened survival period and a decrease in the body weight of infected guinea pigs on a scorbutic diet, and De Savitsch [620] found smaller lesions and a greater increase in weight in animals inoculated with tubercle bacilli and fed sources of ascorbic acid than in the inoculated and untreated controls. Russell, Read and Rouse [889] have shown that compared with controls there ... d scorbutic ... ion. They ... was slightly greater in the organs of control animals receiving adequate ascorbic acid. These observations are in agreement with the early laboratory studies on scorbutic guinea-pigs, which often died of tuberculosis. McConkey and Smith [621] concluded that the administration of tuberculous sputum to guinea-pigs was not the sole cause of intestinal ulcers. Their control animals given adequate ascorbic acid developed ulcers in only two instances, compared with twenty-six in the group suffering from ascorbic acid deficiency. Steinbach and Klein [809] have claimed that the daily injection of ascorbic acid into tuberculous guinea-pigs increased their tolerance to repeated large doses of tuberculin. Similar observations were made by Birkhaug [150], who reported that ascorbic acid inhibits the tuberculin reaction in tuberculous guinea-pigs. He also records that large doses of the vitamin caused a significant increase in weight and reduction in the tuberculous lesions of guinea pigs infected with tuberculosis. The histochemical studies of Tonutti [102] show that re-infection of experimental animals after a previous sensitization results in the accumulation of cells in the lung alveoli laden with ascorbic acid. In addition the bronchioles are often seen to be choked with desquamated cells saturated with ascorbic acid. Such studies explain why the plasma and urine concentration of ascorbic acid are lower in tuberculosis.

It is doubtful if the administration of ascorbic acid beyond the normal requirements is of any benefit to tuberculous patients, although its use has been advocated [625–632]. Most of the workers who claim that it has a beneficial effect speak of general improvement, but produce no evidence in the form of radiological changes, gain in weight or lowering of the sedimentation rate. Heise and Martin [624] claim that the administration of ascorbic ... ment

... radiological improvement. McConkey [1012] thought ... tomato juice were helpful in preventing laryngeal tuberculosis as a complication of pulmonary tuberculosis. To what extent vitamins A and D and the fatty acids in the oil might play a part is unknown. Erwin and others, at

Liverpool [636], and Kaplan and Zonnis [850] saturated tuberculous patients with ascorbic acid for six months, but failed to observe any significantly favourable results, as judged by the usual clinical criteria, when compared with control patients.

Pertussis. Ascorbic acid in a concentration of 80 mg per 1,000 ml inhibits the growth of *Hæmophilus pertussis* [649]. Although such a concentration cannot be reached in the plasma, it can in the white cells [102]. Animal experiments also suggest that the virulence of *H. pertussis* is reduced if injected with ascorbic acid. Following these observations several enthusiastic but uncontrolled reports appeared on the value of ascorbic acid in the treatment of whooping cough [650–652]. The administration of 100 to 300 mg daily was said to reduce the number of whoops considerably and to shorten the duration of the disease from weeks to days [651]. A carefully controlled study by Gardner [651] at Great Ormond Street Hospital for Children failed to confirm these observations. Stringent criteria of diagnosis were recognized: recovery of *H. pertussis* from a cough plate, a paroxysmal cough, lymphocytosis and a sublingual ulcer. The number of paroxysms was counted and the weight of the children carefully controlled. The duration of illness and the gain in weight did not differ significantly in the treated and control group.

Respiratory Infections. Glazebrook and Thomson [816] state that the duration of an attack on tonsillitis is less in adolescents treated with ascorbic acid than in controls not receiving the vitamin.

Good results have been reported in the treatment of the common cold with large doses of ascorbic acid, e.g. 300 to 1,000 mg daily [662, 663, 741]. These trials were uncontrolled and based on the subjective judgments of the patient. In a trial conducted among Dutch factory workers the prophylactic use of ascorbic acid and quinine was stated to reduce the incidence of the common cold [844]. However, the number of working days lost was the same in the treated and untreated group. Carefully controlled observations by Cowan, Diehl and Baker [742], Glazebrook and Thomson [816], Brown [506] and several Scandinavian workers [836, 841, 857] have shown that there is no evidence that ascorbic acid has any statistically significant effect in preventing the common cold or affecting its duration. Dahlberg, Engel and Rydin [836] carried out a mass experiment on 2,500 Army conscripts, one half receiving 200 mg of ascorbic acid, the other half acting as controls. No difference was noted in the frequency or duration of colds, fever, endurance test or diseases of any description in the two groups.

Rheumatic Fever. The ascorbic acid excretion is lower than normal in patients with rheumatic fever as in other acute infections [435, 467, 468, 638, 639]. Rinehart [638] noted in 1934 that guinea-pigs infected with β streptococci developed an arthropathy that could be prevented by ascorbic acid. As the ascorbic acid excretion in patients with rheumatic fever is low Rinehart argued that the disease might result from the combined effect of infection and ascorbic acid deficiency. The experimental work of Rinehart has been confirmed but his conclusions doubted [640, 641]. Schultz and others [640, 643–645] pointed out that the lesions in Rinehart's guinea pigs only superficially resembled those of rheumatic fever. He argued correctly that the low ascorbic acid excretion in rheumatic fever is the result and not the cause of the infection, which he found to be uninfluenced by administering large doses of ascorbic acid [643]. Abt and his co-workers [152] found the clinical course of acute rheumatic fever uninfluenced by the administration of ascorbic acid. During treatment the patient with rheumatic fever may excrete more ascorbic acid than the average febrile patient because salicylates increase excretion (p. 439). This has been shown by Keith and Hickman [646]. Salicylates increase the excretion of ascorbic acid by depleting the supplies in the adrenals [622].

In 1950 Massell and his co-workers [623] published a preliminary report

probably not directly related to gingivitis. Stuhl and Buchanan state that the effects of local treatment (gentian violet two per cent.) are enhanced by the administration of ascorbic acid. Buchanan states that ulcerative gingivitis does not clear up with normal dental treatment and is progressive unless local treatment is supplemented by treatment with 700 mg. of ascorbic acid daily until the patient is saturated.

The observations of Roff and Glazebrook, Campbell and Cook, Stuhl and Buchanan have been criticized by King [536] who states that the grading of lesions was not described in sufficient detail and that little indication was given of criteria of cure.

There is considerable difference of opinion on the ætiology of ulcerative gingivitis (ulce [illegible] Vincent's disease, "trench mouth," fuso spirochæt[illegible] doubts whether it is associated with ascorbic [illegible] ondition may clear up with local treatment alone and the use of ascorbic acid without the latter gives variable results. Pincus observed two hundred men with ulcerative gingivitis and states that many were getting enough ascorbic acid. Cuthbert and Williams [986] also failed to show any degree of ascorbic acid subnutrition in sailors suffering from ulcerative gingivitis. The level of ascorbic acid nutrition was no worse in these men than in non-infected healthy seamen. They found that the administration of the vitamin did not accelerate cure of the gingivitis and they were inclined to think that the chief factor in the spread of the disease is contagion, e.g. due to "French kissing," inadequate washing of crockery and dirty oral habits. The view of Ministry of Health dentists is that "there is ample evidence that ascorbic acid therapy is ineffective" in the treatment of ulcerative gingivitis [985].

Kent [983] believes that much confusion over treatment has resulted because there are two clinical entities, latent scorbutic gingival ulceration and ulceromembranous gingivitis or Vincent's disease. In the former local therapy fails and the response to ascorbic acid is dramatic; in the latter local therapy is necessary. Linghorne and his co-workers [554] found no improvement in cases of gingivitis treated with large doses of ascorbic acid for five months. But they also state that gingivitis is developed more frequently in subjects with a low blood ascorbic acid than in those with high levels. When the gingivitis was cleared up the provision of 75 mg. ascorbic acid daily appeared to have a delaying effect on recurrence. Yet these workers did not consider gingivitis scorbutic because they remarked that "the histological appearances of the gingival tissues in no way resembled the changes seen in scurvy."

MacDonald [979] noted that there was a high incidence of bleeding gums and gingivitis in naval ratings. He was unable to show that it was related to ascorbic acid deficiency and the condition cleared up with local treatment. Ungley and Horton [1016] made similar observations on naval patients with sore and bleeding gums, in whom scurvy or "subscurvy" was absent. Local causes such as infection and calculus, were sufficient to account for the gum condition, and both ascorbic acid and nicotinic acid were ineffective. Day and Shourie [958] found that ascorbic acid was ineffective in the treatment of gingivitis and associated conditions in Indian children. Lesson and his co-workers [490] treated one hundred and twenty-nine subjects with gingivitis with 25 to 125 mg. ascorbic acid daily, but observed no improvement after three months' treatment. Stammers [951] strongly advocates local treatment in ulcerative gingivitis. He finds ascorbic acid and nicotinic acid therapy disappointing, with a response rate of only eighteen per cent. Recently Stamm, Macrae and Yudkin [918] found that twenty per cent. of a group of nearly three thousand R.A.F. personnel suffered from bleeding gums, but no greater improvement was obtained by treatment with ascorbic acid than with inert control tablets. Those with "sponginess" as well as bleeding of the gums showed no more improvement after taking ascorbic acid than when

given control tablets These workers concluded that the incidence of bleeding gums is not related to ascorbic acid deficiency

These conflicting views on ascorbic acid and gingivitis are undoubtedly due to confusion over the ætiology and symptomatology of inflammatory conditions of the gums and the absence of established standards for the normal gum Many investigators do not state the exact type of lesion treated and the criteria for assessing recovery or for assessing vitamin deficiency

The recent observations of Farmer and his colleagues [864] suggest that there is probably no connection at all between ascorbic acid deficiency and dental lesions excluding of course frank scurvy They kept volunteers on a scorbutic diet for five months but failed to observe any changes in the gums teeth or bone of the jaw as observed with the naked eye radiologically or with the biomicroscope The plasma ascorbic acid and the ascorbic acid in the white cell layer were zero These observations suggest that the oral conditions ascribed to ascorbic acid deficiency by some writers are in reality due to pre existing caries and gingivitis or improper dental hygiene and are not related to ascorbic acid deficiency

It is stated by Campbell and Cook [853] that the oral administration of ascorbic acid to patients before and after dental extractions plays an important role in the healing of the wounded gum tissue and the absorption of the alveolar bone margins They also report that pain and bleeding particularly persistent hæmorrhage after extractions are considerably reduced The patients were given 300 mg of vitamin daily until saturated and then 100 mg daily as a maintenance dose Persistent hæmorrhage was treated with 500 mg of the vitamin There is no evidence that such large doses as this were necessary

Hæmatology In scurvy erythropoiesis is depressed in the bone marrow the anæmia usually responds to treatment with ascorbic acid (p 416) Recent work in this country has drawn attention to the increased incidence of anæmia during the war particularly among pregnant women An inadequate intake of iron has been suggested as the cause but it may be that ascorbic acid deficiency plays a part as well [557] Israels [557] suggests that any patient whose anæmia does not respond to iron should be given ascorbic acid Davidson and Donaldson [794] treated hypochromic anæmia in children with iron and observed that daily supplements of 25 mg of ascorbic acid had no effect in raising the hæmoglobin level Dyke and Della Vida [734] state that a number of pernicious anæmia patients in spite of their maintenance dosage of liver showed a progressive fall in the red blood count This was checked by giving 100 mg of ascorbic acid daily for a month the red blood count increasing by 1 000 000 per c mm These observations suggest that in pernicious anæmia even with ample dosage of liver extract hæmopoiesis is subnormal if there is any degree of ascorbic acid deficiency

The prompt response of the petechial hæmorrhages of scurvy to the administration of ascorbic acid suggested its use in hæmorrhagic conditions other than scurvy There have been reports on its use in the treatment of purpura [685 694] but these early enthusiastic reports have not been substantiated by later workers [695 702] Scarborough [969] treated fifteen patients with purpura with ascorbic acid without success and Davis [558] from an analysis of 1 200 cases of Schonlein Henoch purpura concluded that ascorbic acid was of no value Ascorbic acid is also ineffective in hæmorrhagic states [717 719]

It has been claimed that hæmaturia is a manifestation of ascorbic acid deficiency and responds to treatment with the vitamin [693 709 711] There is no evidence that hæmaturia is associated with ascorbic acid deficiency except in cases of scurvy The vitamin has no effect in controlling hæmaturia due for example to renal lesions [712] Claims have been made for the effectiveness of ascorbic acid in the treatment of leukæmia [720 722] but they have not been confirmed [575 721 724]

Vogt [721] Carrie [723] and Ellis [618] state that leucopenia from irradiation with X-rays can be corrected with ascorbic acid. Ellis states that a sufficiently large dose can prevent the fall in the white count that follows irradiation. Field and Rekers [587] have been unable to confirm this in dogs. There is apparently a fall in the ascorbic acid of the plasma and tissues of animals exposed to roentgen irradiation [622]. Kalk [724] claims that ascorbic acid is effective in the treatment of granulocytopenia in doses of 500 to 1,000 mg daily.

Lian [1008] and Deeny and his co-workers [963] showed independently that familial idiopathic methæmoglobinæmia, one of the "inborn errors of metabolism," and characterized by a permanent slaty blue colour of the skin and methæmoglobin in the blood, responds to treatment with 300 to 400 mg of ascorbic acid daily and sodium bicarbonate. The general health in this disease does not suffer, as there is sufficient functioning oxyhæmoglobin. Treatment with ascorbic acid or methylene blue results in disappearance of the bluish colour of the skin, which becomes normal and remains so as long as treatment is maintained. Ascorbic acid reduces methæmoglobin to hæmoglobin. Normally methæmoglobin reduction in erythrocytes takes place through the oxidation of triose phosphate and lactate, and the system requires co-enzyme I [602]. The cause of idiopathic methæmoglobinæmia is congenital absence of a factor in the erythrocyte mechanism that in normal red cells rapidly reduces methæmoglobin [603]. The observations of Lian and Deeny have been confirmed by a number of other workers [595–597, 600, 603].

The studies of Barron [101] suggest that ascorbic acid might play a part in maintaining the hæmoglobin of the blood at a normal physiological level. He found that in polycythæmia induced by administering cobalt to animals the red cell count could be reduced by ascorbic acid. A fall in the hæmoglobin also occurs [642]. Deeny [726] observed that ascorbic acid and sodium bicarbonate caused a marked fall in the red cells count in two patients with polycythæmia vera. Kandel and LeRoy [727] had previously failed to find any beneficial effects from giving ascorbic acid in this condition.

Ascorbic acid protects erythrocytes *in vitro* from the hæmolytic action of hypotonic solutions of saline [807]. If this is confirmed *in vivo* ascorbic acid may be of therapeutic value in the treatment of patients having increased fragility of the red cells.

Ascorbic Acid in Dermatology. During his experiments on induced scurvy Crandon [68] noted that after a hundred and thirty four days on a diet practically free from ascorbic acid small hyperkeratotic papules began to develop over the buttocks and posterior aspects of the calves. Noticeable fragmentation of the hairs and marked dryness of the skin were also noted. It was proved that these skin lesions were not the result of vitamin A deficiency, as 30,000 units of this vitamin were being taken daily. The administration of ascorbic acid rapidly cured these skin lesions.

It has been suggested that ascorbic acid is intimately connected with pigment formation and pigmentary disorders of the skin (p. 430), in which it is encountered for the most part in the basal layer of the epidermis and in hyperpigmented structures. The formation of pigment in the skin depends on the ability of the melanoblasts to produce it, probably through the oxidation of certain organic substances, including L 3 4-dihydroxyphenylalanine ("dopa"). All pigment producing cells stain black with *dopa reagent*. According to Szent Györgyi [231] ascorbic acid inhibits the formation of pigment ... the oxidation of phenolic substances, such as *dopa*,
and by
be able
been verified by *in vitro* experiments [232–234] ...
pigmentation of the nipples and areolæ of castrated guinea-pigs receiving œstrogens was uninfluenced by giving ascorbic acid [729]. Cornbleet [730]

found that human skin contained less ascorbic acid after ultraviolet irradiation He further observed that a section of skin from a negro injected with ascorbic acid reacted more to silver nitrate than a section of skin from a white person who had received a similar amount of ascorbic acid He inferred from this that the pigment fixed the ascorbic acid in the skin

If ascorbic acid has an inhibiting effect on pigment production it might explain the hyperpigmentation that occurs in scurvy and Addison s disease It is stated that the pigmentation of this condition is diminished by the administration of the vitamin [235 237 23) 872]

Ascorbic acid has been recommended in the treatment of a number of skin conditions such as psoriasis [508 731 733] urticaria [736] erythema multiforme [739] and infantile eczema [740] The evidence is not impressive A low excretion of ascorbic acid has been noted in lupus vulgaris and erythematosus but administration of the vitamin had no effect on the course of the disease [737 738] Ascorbic acid is said to be of value in the prevention and treatment of poison oak dermatitis [475]

Lever and Talbott [744] examined the blood ascorbic acid in a hundred and eighty one patients suffering from various skin lesions including dermatitis psoriasis urticaria lupus vulgaris lupus erythematosus eczema exfoliative dermatitis pemphigus and acne vulgaris The results were compared with those of sixty eight apparently healthy persons Great variations were found but there was no significant correlation between the blood ascorbic acid and the development of the skin lesions and large amounts of the vitamin were given to eighteen patients showing low levels for ten weeks but without any improvement in their clinical condition Braestrup and Hansen [745] observed that ascorbic acid had no beneficial effect on the clinical condition of patients with skin lesions

The follicular lesions commonly seen in this country are probably those of keratosis pilaris and have no direct association with a deficiency of ascorbic acid or vitamin A [476 492]

Ophthalmology According to Friedenwald ascorbic acid plays an important part in the nutrition of the ocular tissues and is essential for the secretion of aqueous humour [962] According to him ascorbic acid is secreted into the aqueous humour by the ciliary body and this is the source of the ascorbic acid in the lens In several animal species the aqueous humour contains twenty to fifty [illegible]
This high concentration [illegible]
acid is more concentrate [illegible]
e g the retina vitreous humour the ciliary processes the cornea and the lens contain high concentrations of ascorbic acid [489 494 601 747 962] The concentration of ascorbic acid is greatest in the superficial layers of the cornea [653] and falls after injury [494] Lyle and McLean [755] treated patients suffering from the following inflammatory conditions of the cornea with ascorbic acid dendritic corneal ulcer disciform keratitis sclerosing keratitis superficial punctate keratitis post vaccinial keratitis and corneal ulceration all of which occurred as complications of eye injuries in airmen One thousand mg ascorbic acid was given intravenously as well as local treatment Improvement was stated to be dramatic and was attributed largely to the treatment with ascorbic acid As local treatment was given as well it is impossible to assess the part played by ascorbic acid Similar observations were made by Summers [659] who used penicillin as well so that the part played by each substance could not be evaluated

Campbell Ferguson and Garry [655] made superficial and deep heat injuries on the corneas of normal and ascorbic acid deficient guinea pigs They found that the healing of superficial lesions was not impaired by ascorbic acid deficiency but the deeper lesions involving the substantia propria of the cornea healed more slowly in deficient guinea pigs Even after healing was complete structural weakness of the wound persisted for as long as three

post mortems on twenty eight patients dying several days after perforation of a peptic ulcer or after operations on the stomach In those patients with a very low level of ascorbic acid nutrition wound infection was common and collagen formation which is essential for wound repair was either poor or absent Microscopically the wounds were like those of scorbutic guinea pigs There is no reason to believe that ascorbic acid is ætiologically related to peptic ulceration as was suggested by Smith and McConkey [676] and Roe and others [806]

The production of experimental peptic ulcers in dogs by cinchophen can be prevented by the administration of ascorbic acid [677] This may act by detoxifying cinchophen and it does not necessarily follow that ascorbic acid has any effect on the prevention or healing of human peptic ulcers Some workers have claimed that its administration promotes their healing but the evidence for this is very slender [776 777]

According to Abt Chinn and Farmer [473] the absorption of ascorbic acid is defective in most patients with achlorhydria It is unlikely that hydro chloric acid is needed for the absorption of ascorbic acid as the administration of alkalis does not seem to interfere with absorption [277 332 491]

Obstetrics The requirements of ascorbic acid during pregnancy and lactation have been mentioned previously (p 447) Some workers have stated that the diet of most pregnant women is deficient in ascorbic acid but this view is based on excretion or blood studies which have probably led to false conclusions [874 998] For example Williams and Fralin [874] make the sweeping statement that only two per cent of women consume an adequate diet in pregnancy

Javert and Stander [989] state that lack of vitamins C and K may be a factor in the pathogenesis of threatened and spontaneous abortion and ante partum bleeding Of seventy nine patients with threatened spon taneous or habitual abortion sixty nine per cent had a lower plasma ascorbic acid than normal controls King [703] also noted low blood ascorbic acid levels in cases of inevitable and complete abortion Skin ecchymosis epistaxis bleeding gums and vaginal bleeding were common but these symptoms are not necessarily associated with a deficiency of ascorbic acid only two of them are seen in scurvy Javert and Stander treated thirty three patients with threatened abortion with ascorbic acid vitamins E and K minerals trace elements and progesterone and they claim that the incidence of abortion was reduced in comparison with controls So many preparations were used that it is impossible to ascribe the fall in the abortion rate to any one of them Other workers state that ascorbic acid given either prophy lactically or for treatment prevents threatened abortion progressing to inevitable abortion [781 783] The reports are unconvincing because of lack of adequate control observations It is well known that women who have previously aborted often become pregnant again and proceed to term with the delivery of a healthy live infant without any specific form of treat ment

Kappeli [780] observed that ascorbic acid increases uterine tone in the isolated guinea pig uterus producing a slow steadily increasing contraction which either becomes rhythmic or tetanic According to this observer it produces either relaxation or a tetanic contraction in the human uterus He states that in doses of 100 to 250 mg given orally or intramuscularly it prolongs the uterine contractions caused by pituitary extract The effect begins in five to ten minutes after intramuscular injection and lasts for forty five to sixty minutes This work has not been confirmed

Surgery Wound Healing, Burns The importance of ascorbic acid for the healing of wounds has been previously mentioned (p 410) Many workers have demonstrated that surgical patients often have low ascorbic acid reserves and that for some days after operation there is a considerable drop in the ascorbic acid in the blood and urine [786 787 788] The stores of

ascorbic acid are likely to be low in patients undergoing operations for gastric lesions because of dietary restrictions. Lund [786] found that many gastric cases coming to operation were bordering on scurvy. When non radical operations were performed more complications and deaths were observed among the patients with low reserves of ascorbic acid than among others. According to Bartlett and his co workers [787] the magnitude of the surgical procedure is related to the post-operative ascorbic acid reserves; a parenteral dose of 1 000 mg. of ascorbic acid disappears from the blood stream far more rapidly after operation than before. Bartlett and his co-workers [852] have also shown that a sufficient depletion of ascorbic acid will decrease the tensile strength of healing wounds in the skin and fascia of human beings. According to them a fasting plasma ascorbic acid less than 0.2 mg. per 100 ml. is likely to interfere with wound healing, although Bourne [925] has been unable to confirm this. They state that in the presence of adequate ascorbic acid the ascorbic acid content of healing fascial scars is much greater than that of healing skin scars. The local application of ascorbic acid to wounds does not accelerate healing [85].

According to Hunt [77] ascorbic acid deficiency is a cause of wound eventration, the incidence of which was reduced in a series of surgical cases by seventy-five per cent after saturating all the patients with ascorbic acid before operation. He also states that leakage from suture lines occurred only once in two and a half years after he adopted the practice of saturating all his patients with ascorbic acid, and that when wounds failed to heal in saturated patients gross infection, hæmatoma formation or ischæmic necrosis could often be incriminated as the cause. Hunt examined sections of abdominal incisions, sutured ulcers and gastro-intestinal anastomoses from patients who had succumbed after abdominal surgery. In those cases where collagen was poorly formed and cellular proliferation scanty there was a history of a low intake of ascorbic acid (Figs. 174 and 175). The microscopical appearances resembled those seen in guinea pig scurvy (Figs. 176 and 177). Histological examination of the wounds of those patients whose intake of ascorbic acid was considered adequate showed normal collagen formation and cellular proliferation. Kraybill [909] observed seven cases of abdominal wound disruption; in each case the blood ascorbic acid was zero. Hunt also believes that the absorption of catgut ligatures may be delayed and that post operative peritonitis may be more common among patients with low ascorbic acid reserves.

Lund and Crandon [813] studied the correlation between the ascorbic acid nutrition and the post operative healing of abdominal wounds. While they state that post-operative wound eventration occurs more readily in patients with low ascorbic acid reserves, they think that mechanical factors are more important. Hypoproteinæmia, infection and bad suturing can also be causes. They consider that a daily intake of 20 mg. ascorbic acid adequate for wounds to heal easily and well. Pijoan and Lozner [873] consider that a daily intake of 12 to 25 mg. of ascorbic acid, which is adequate to maintain a level of 25 mg. per 100 ml. in the white cell layer (p. 470), is sufficient to ensure adequate wound healing and collagen formation. They base this view on observations on a subject whose daily ascorbic acid for twenty months averaged 16 mg. The plasma ascorbic acid was never more than 0.2 mg. per 100 ml. An experimental wound made in the back showed normal healing ten days after, when a biopsy was done. Carney [706] noted a very large scatter (0.1 to 2.4 mg. per 100 ml.) in the plasma ascorbic acid levels of one hundred surgical patients. Those who had repeated burst wounds or whose wounds failed to heal showed plasma levels varying from 0.5 to 1.44 mg. per 100 ml. with a mean level of 0.93 mg. He concluded that there was no relationship between plasma ascorbic acid levels and wound healing. This conclusion is probably correct because it is the level of ascorbic acid in the white cells that reflects the tissue reserves and not the plasma level (p. 470).

ASCORBIC ACID AND WOUND HEALING

FIG. 174. Ascorbic Acid and Wound Healing. Cross section of a sutured skin incision of a patient given 2,600 mg. of ascorbic acid in divided doses after operation for perforated duodenal ulcer.

FIG. 175. Ascorbic Acid and Wound Healing. Cross section of a sutured skin incision of a patient grossly deficient in ascorbic acid. Stain, silver impregnation. The opposed edges of the corium are stretched apart and the gap filled with a mass of proliferative fibroblasts producing immature *precollagen only*. In other regions where the scar was more stretched the gap was filled with *œdematous granulation tissue* with little precollagen and no collagen.

ASCORBIC ACID AND WOUND HEALING

Fig 176. Ascorbic Acid and Wound Healing Control. Cross section of the

muscle by fibrous tissue

Fig 177 Ascorbic Acid and Wound Healing

According to Bartlett and his co-workers [852] normal wound healing may result, in spite of a low content of ascorbic acid in the plasma before operation, if adequate vitamin is given post operatively. They have also shown that focal infection distant from the healing scar does not alter its ascorbic acid content or its tensile strength [954] Local wound sepsis, however, reduces the tensile strength of a wound and interferes with its normal healing. The local use of sulphanilamide does not reduce the tensile strength of a wound scar nor does it retard healing Analysis of scar tissue also reveals that sulphanilamide applied locally does not diminish its ascorbic acid content or increase the need of ascorbic acid in the production of a normal strong cicatrix In the presence of considerable ascorbic acid deficiency local sulphanilamide does not promote wound healing nor does it adequately control wound infection. Bartlett and his co workers [954] noted that in scorbutic animals the production of focal walled off abscesses distant from the operative scar was followed by ultimate infection of the scar with the same organism They call attention to the possibility that maximal tissue saturation with ascorbic acid may increase tissue resistance to infection as well as promote optimal conditions for wound healing

From a study of thirty cases before and after operation Wildbolz [791] concluded that there was little evidence that ascorbic acid deficiency had any relation to post-operative complications The administration of ascorbic acid to all operation cases during the course of a year reduced the incidence of post-operative complications from 24 2 to 19 7 per cent , but this reduction was not statistically significant.

On account of the necessity of ascorbic acid for wound healing massive doses of ascorbic acid are often given to surgical patients pre- and post-operatively, e g 1,000 mg for nine to ten days [75] It is possible that much less than this is adequate Hunt [77] suggest 100 mg daily, but 50 mg daily is probably enough in view of the observations of Lund and Crandon [813] and Pijoan and Lozner [873] If there is any reason to suspect impaired absorption or utilization the amount should be increased and given parenterally if necessary

Hunt [77] suggests that the administration of ascorbic acid to surgical patients should be particularly considered under the following circumstances (*a*) when clean and quick healing of a wound is desired ; (*b*) in major operations , (*c*) when a hollow viscus has been opened , (*d*) when post operative complications are anticipated ; (*e*) when there is a history of insufficient intake of ascorbic acid , (*f*) in all cases of serious injury , (*g*) in patients with a history of vomiting over long periods ; (*h*) in cases of obstructive gastro intestinal lesions and hypermotility of the small intestine , (*i*) in syphilitics and alcoholics , and (*j*) in patients receiving fluids such as glucose or saline intravenously or per rectum over a prolonged period

The importance of an adequate intake of ascorbic acid for the healing of wounds is recognized by the Medical Division of the National Research Council, U S A , who recommend a daily oral intake of 75 mg in all wounded and burnt men in the Services until recovery is complete [956]

According to Evans [953] skin grafts do not take readily in subjects with a low level of ascorbic acid nutrition

Levenson and his co-workers [704] studied six patients with severe surgical conditions and observed very low ascorbic acid reserves The patients were average hospital patients suffering from injury, hæmorrhage or infection Beattie [706] also mentions the fall in ascorbic acid excretion after injury A series of patients admitted for injuries or burns received 500 mg ascorbic acid daily and no trace of the vitamin was found in the urine for five days

Burns Beattie [706] found that even when 500 mg of ascorbic acid was administered daily to patients with burns no vitamin was excreted until the twenty-third day, in contrast to normal operation cases that excreted 50 to 100 mg of ascorbic acid by the fifth day It is probable that some part

of the increased demand for ascorbic acid in injury and burns may come from the adrenal cortex (p 430) Certainly there is a fall in the ascorbic acid level in the adrenal cortex of burned animals [707 708] Lund and his colleagues [713] consider that large doses of ascorbic acid, e g 1 gram daily, thiamine, riboflavine and nicotinamide are needed by severely burned patients A similar conclusion was reached in a study of patients with hæmorrhagic shock, traumatic injuries and infection

Shock. Loss of hæmoglobin in acute hæmorrhage and circulatory failure in shock both lead to a considerable diminution in the oxygen supply to the tissues In order to improve the transfer of oxygen from the blood to the tissues in cases of hæmorrhage and circulatory failure, Stewart and his associates [795] suggested the intravenous injection of ascorbic acid Five control cats were bled until the blood volume was reduced to half, the blood pressure fell considerably and the animals died within an hour Sixteen other cats were treated similarly, but they also received sodium ascorbate intravenously in doses from 200 mg per kilo of body weight to a dose of 2 grams In all cases the blood pressure rose and the survival period was much longer than in the control animals In those animals that survived, a striking feature was the rapid reappearance of good pulse pressure and improvement in respiration after the injection of ascorbic acid De Pasqualini [715] has also prevented hæmorrhagic shock in guinea pigs by pre treatment with ascorbic acid The work of these investigators suggests the use of ascorbic acid in cases of accidents and injuries involving hæmorrhage The dosage they suggest is 3 grams of sodium ascorbate The observations of Lucas [804] confirm Stewart's suggestion that ascorbic acid secures a more adequate supply of oxygen for the tissues Lucas has shown that ascorbic acid administered intraperitoneally increases the resistance of mice and rats to low oxygen tensions It has also been demonstrated that low concentrations of ascorbic acid increase the rate of respiration of isolated liver tissue [845]

Ungar [946] using a standard traumatizing technique in guinea pigs, rats and mice, has shown that the injection of ascorbic acid within fifteen minutes after trauma reduces post traumatic mortality in these animals The minimum effective dose was 100 mg per kilo of body weight This effect is independent of the vitamin action of ascorbic acid as it can be produced after oxidation, moreover rats and mice, the experimental animals used, do not suffer from ascorbic acid deficiency as they synthesize their own vitamin

McDevitt and her co workers [945] showed that scorbutic guinea pigs are more susceptible to shock than normal animals and that repeated trauma in animals with a normal intake of ascorbic acid "conditions" them to traumatic shock It was found that massive doses of the vitamin immediately following trauma increase the survival time of animals but do not lower the mortality rate

Anæsthesia Animal experiments suggest that tolerance to anæsthetics may be increased by the administration of ascorbic acid The urinary excretion is increased in the dog, rat and guinea pig after ether anæsthesia, and analysis of the adrenals, kidneys, liver and ovaries shows a fall in the ascorbic acid content of these organs after ether, chloroform or ethyl chloride anæsthesia [213, 796] Guinea pigs treated with 50 to 100 mg of ascorbic acid show an increased tolerance to anæsthetics, they can withstand anæsthesia for a longer period than control animals that have not received the vitamin [213]

Beyer, Stutzman and Hafford [915] have shown that after anæsthesia with cyclopropane, ether divinyl ether (vinesthene) and chloroform, the plasma ascorbic acid rises for the first seven hours subsequently falling in twenty four hours below the original level This would account for the increased excretion after anæsthesia found by others Beyer and his co

workers have also shown that in animals suffering from ascorbic acid deficiency anæsthesia can be induced more rapidly, the animals are more depressed by a given dose, and they recover more slowly than healthy control animals treated with 30 mg of ascorbic acid daily. If these data can be transferred to clinical anæsthesia, they suggest that large doses of ascorbic acid might be helpful before an anæsthetic is administered

Psychiatry. In view of the dietetic idiosyncrasies and increased physical activity of some mental patients and the low ascorbic acid content of most institutional diets it is not surprising that a considerable degree of ascorbic acid deficiency has been found in such patients [495–498] Some investigators have found low ascorbic acid levels in patients suffering from alcoholic and senile psychoses [495, 498, 503] There is no evidence of any direct causal relationship between ascorbic acid deficiency and mental disorder If a deficiency is found, it is usually due to malnutrition One would expect, however, by improving the nutritional status of a patient to improve the general condition and even mental outlook of the patient Minski and Constantine [797] found no correlation between the mental condition and the level of ascorbic acid in psychotic and psychopathic patients, nor did they observe any material change in the mental state of patients who had been treated with ascorbic acid Stoltz [842] failed to observe any signs of ascorbic acid deficiency in patients with schizophrenia Cranswick and Hall [501] gave ascorbic acid and desoxycorticosterone (DOCA) to thirty seven patients with mental disorders. Those with a history of less than one year showed euphoric reactions and some beneficial effects, which were attributed to the liberation of a cortisone-like substance in the body. Similar observations were made by Bourne [774]

Cardiovascular Disease. Diuretic Action. Abbasy [801] has observed that ascorbic acid in doses of 700 mg. has a specific diuretic effect. This was noted during investigations on the excretion of the vitamin after giving test doses, and confirmed by using control subjects It is claimed that the diuretic property of ascorbic acid may be of some use in cases where a slow and progressive dehydration of the body is desired, particularly as it is safe and unlike mercury diuretics does not damage the kidney Evans [802] studied the diuretic effect of ascorbic acid in eight cases of heart failure and one of œdema of unknown origin In doses of 500 to 300 mg a day it produced a diuresis in all patients, as judged by excess of urinary output over fluid intake, its diuretic effect was greater than that of digitalis but less than that of theobromine, diuretin and ammonium chloride The diuretic effect of ascorbic acid has also been observed by Lueg and Hammann [803], who state that it can be used to reinforce the mercurial diuretics, such as mersalyl, and actually increases their tolerance, since toxic effects on the kidney and liver are not so frequently observed when they are given with ascorbic acid

Shaffer [944] noted that ascorbic acid in doses of 500 mg produced a diuresis in patients with cardiac decompensation The actual increase on a standard fluid intake of 1,500 ml was from 250 to 1,000 ml, in seventy-two hours When given intravenously it had no appreciable effect, possibly due to its rapid elimination by the kidney In later publications with others Shaffer [179, 751] states that 100 mg of ascorbic acid three times daily enhanced the diuretic effect of the organic mercurial drugs and diminished their toxicity Anderson [752], using a self-retaining catheter, found that ascorbic acid had no diuretic action if the subject is receiving intravenous saline at the same time

Croft, Jones and Richter [935] found that neither aneurine nor ascorbic acid deficiency is a significant factor in producing fatigue and other symptoms in patients with the effort syndrome

Holtz [784] advanced the theory that histamine was formed *in vivo* and *in vitro* by the action of ascorbic acid on histidine, and Block and Pinosch [789] found that there was an increase of histamine in the lungs of guinea pigs

following the administration of histidine and ascorbic acid This suggested the use of histidine and ascorbic acid in the treatment of peripheral vascular disease the continual and slow liberation of histamine producing vasodilation Wirtschafter and Widmann [790] claimed that injections of histamine and ascorbic acid improved the blood supply in patients with peripheral vascular disease Friedell and his co workers [792] treated a group of twenty five patients with impaired circulation of the lower extremities due to arterio sclerosis obliterans they had been selected for amputation Treatment consisted of injections of 5 ml of four per cent histidine every six hours or oral administration of 2 grams of histidine three times daily and 100 mg of sodium ascorbate subcutaneously with each dose of histidine and 600 mg of ascorbic acid orally each day as well These workers claimed that the pain of arteriosclerosis obliterans was relieved and circulatory studies with radio active phosphorus indicated an improvement Weisman and Allen [793] failed to observe any improvement in digital blood flow after administering histidine and ascorbic acid to patients with occlusive arterial disease nor *were ischæmic pain relieved or the ischæmic lesions improved*

Ascorbic acid in large doses has a hypotensive effect on normal blood pressure in the rat and on hypertension due to large doses of salt renal compression and large doses of desoxycorticosterone acetate (DOCA) [743 810] It has not been used for this purpose clinically

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CHAPTER VII

VITAMIN D

THE ANTIRACHITIC OR CALCIFYING VITAMIN

SEVERAL different substances are now known to be antirachitic the term vitamin D is used to embrace them all The most important are

Vitamin D_2 or Calciferol This seldom occurs naturally but is manufactured artificially by irradiating or activating the vegetable sterol ergosterol Viosterol is a name chiefly used in the United States for unpurified irradiated ergosterol it has little meaning as the amount of calciferol in viosterol varies greatly according to the method of irradiation employed

Vitamin D_3 This is the most important naturally occurring vitamin being formed in the skin by the action of the sun on the animal sterol 7 dehydrocholesterol

Vitamin D_1 is a name which is no longer used having been given to a substance which was later found to be an addition compound of calciferol and lumisterol

HISTORY

The early history of vitamin D is the history of rickets It is a depressing history a history of perfect clinical observation on the cure of the disease being forgotten again and again for a century and a half

Rickets suddenly became recognized as a definite disease by medical writers in the last half of the seventeenth century the poor had probably been familiar with it for many years The name rickets according to Skeat [1] is an old English word the adjective rachitic was forced into our language by the mistaken desire of lovers of the classics to give rickets a Greek derivation

The first description of rickets is given by Daniel Whistler who in 1645 published his De Morbo puerili Anglorum quam patrio idiomate indiginæ vocant The Rickets This was a thesis presented for his Doctorate of Medicine at Leyden which has led some to say he was Flemish He was however an Englishman educated at Thame and Merton College Oxford His description of rickets written at the age of twenty five was followed four years later by another by Arnold Boate and it was not until 1650 that Glisson [2] a Cambridge man published his account which for some obscure reason is generally said to be the first J Whitaker in 1646 and Thomas Fuller in 1647 had also briefly mentioned rickets in infants A few much earlier descriptions of what were probably rickets or its adult equivalent osteomalacia have been collected [208 245] though these were not recognized as an individual disease but reported as curiosities

The eighteenth century added little to the history of rickets until towards its end when cod liver oil was first used in medicine though apparently in Scotland and Northern Europe cod liver oil had been popular for many years with the peasants as a cure for rickets and other diseases

In 1782 Dr Robert Darley [3] wrote to Dr Thomas Percival an account of his use of cod liver oil which was so highly successful that the poor clamoured for it though its smell and taste were loathsome as it was made by heaping together the livers of the fish from which by gentle putrefaction the oil flows very plentifully Percival [3] recommended peppermint to conceal the taste—advice which a century and a half of further experience has not bettered But it must be admitted that the earliest account of the

value of cod liver oil did not stress its value in rickets; in fact, only two children were reported, the other cases being arthritis or rheumatism.

After this the use of cod liver oil for rickets seems to have spread rapidly in Germany and Holland and from there to France. By the middle of the last century Trousseau [4] was teaching that cod liver oil was the well known and perfect cure for rickets, or when this was too expensive, large quantities of the best fresh butter—suitably concealed in a mixture so as not to shake the confidence of the patient by prescribing such a simple remedy. He also realized the uselessness of vegetable oils, the value of sun, the drawbacks of cereals, and identified osteomalacia as adult rickets.

The last century had also gone far in elucidating the nature of rickets by animal experiments. Jules Guerin [5] in 1838 had produced rickets in puppies to support his theory that rickets was due to the wrong food, and Bland Sutton, fifty years later, had used cod liver oil, milk and crushed bone to cure rickets in lion cubs at the Zoo.

FIG 178. Rickets in Vienna in the post-War famine of 1920. The children, six years of age, show severe rachitic deformities compared with the normally grown child of the same age in the centre. *(See also Fig 188.)*

At the beginning of the present century all this brilliant clinical observation appears to have been largely forgotten, even though Trousseau's book had been translated into English by the New Sydenham Society. In 1912 Sir William Osler was still only vaguely mentioning cod liver oil as useful in rickets. Indeed, there was amazing confusion on the subject. There were three outstanding theories.

One theory was that it was a chronic infective condition like tuberculosis, this being still widely believed on the Continent as late as 1919.

The second theory was Hansemann's "Domestication Theory" which was upheld by the "Glasgow School," so called from the observations of Fergusson and Findlay [7] on rickets in Glasgow in 1918. The theory is well summed up by Hutchison and Shah [8] in their observations on the effect of Purdah in India in 1922: "The most important ætiological factor in the production of rickets is lack of fresh air, sunlight and exercise." But sunlight was never stressed as the one important factor in fresh air and exercise, though Palm [9] had emphasized this in 1890.

Thirdly, the theory was gaining ground that rickets was a deficiency disease. This was due to the work of Edward Mellanby [10], who by 1918 had produced experimental rickets in puppies by feeding them on diets which were deficient in some factor found in certain animal fats. At first this factor was thought to be the same as the growth factor described in 1913 by McCollum and Davis [11] and the "fat soluble A factor," which was necessary for the protection of the eyes—the antixerophthalmic factor—described in 1917 by McCollum and Simmonds [12].

But further work showed that the antirachitic factor and the anti-xerophthalmic factor were different. They did not always occur in the same proportion in all fats tested, one fat might give little protection against xerophthalmia but give good protection against rickets, while another fat might have the opposite effects It was also found that heat and oxidation destroyed more of the antixerophthalmic than the antirachitic factor.

The importance of calcium and phosphorus in relation to rickets was shown by McCollum when extending Mellanby's work to rats, animals which only develop rickets if these two elements are badly balanced in the diet.

In 1919 Chick and her co workers went to study rickets in Vienna during the post War famine The work was originally undertaken to follow up the clinical possibilities of Mellanby's work. It was extended to include the effect of sunlight, and exposure to the radiations from the mercury vapour quartz lamp, because of the observations of Huldschinsky [13] and others, who had definitely proved that rickets was curable by these forms of light. Chick's final report in 1923 [14] showed that both cod liver oil and sunlight, or the mercury vapour quartz lamp, cured rickets, but improved hygiene with neither cod-liver oil nor sunlight was useless In fact, the dietetic theory of rickets was completely confirmed, and the teachings of the Glasgow school in so far as they taught or implied the value of sunlight

The end of the complicated story of rickets, food, and sunlight was unravelled by the work of Hume [15] and many others; though to the last there were unexpected complications, as when rats did not develop rickets because they were themselves supplementing their deficient diets by eating irradiated sawdust in their cages Many experiments proved that ultra-violet irradiation of an animal or of food produced the antirachitic substance, the ultra violet light activating a substance—" the provitamin "—found only in the unsaponifiable fraction of fat

So it was finally shown that animals can either make their own vitamin D by the aid of the ultra violet rays of the sun, or they can get it by eating other animals which have themselves already made it.

CHEMISTRY OF VITAMIN D

All the forms of vitamin D so far investigated are derived from sterols, generally by photochemical reactions They are chemically, but not apparently physiologically, related to the sex hormones, the bufotoxin of toad venom, the digitalis and strophanthus alcohols, and the carcinogenic hydrocarbons [6] The chemistry of the sterols is extremely complex · the following account of the formation and properties of vitamin D_2 and vitamin D_3 is largely taken from the excellent review and papers by Bills [16] For an account of the early work on the chemistry of vitamin D_2 and its preparation in a pure form by English and German workers in the same year (1931), the reader should consult the Medical Research Council's " Vitamins : A Survey of Present Knowledge," published in 1932

Vitamin D_2, or calciferol, was the first of the D vitamins to be fully investigated, though as will be seen when the physiology of vitamin D is discussed, it is as used in medicine an entirely artificial product made from ergosterol—a sterol only found in pl [illegible]

[illegible] ...rol to the action of ultra violet light The absorption spectrum of ergosterol gives bands of maximum [illegible]

[illegible] be produced by irradiating ergosterol for calciferol itself is changed by irradiation, especially by wavelengths shorter than 270 millimicrons.

Ergosterol can be irradiated either as a solid or in solution: the latter is the only satisfactory way, the products of irradiation on the outside of the solid ergosterol apparently protecting the rest of it from the effects of irradiation.

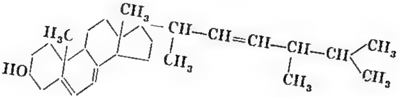
The solvent is important; for instance, if alcohol is used it is difficult to avoid over irradiation, with the result that vitamin D_2 or calciferol is destroyed with the production of the toxic toxisterol in its place. Ether, on the other hand, makes it relatively easy to control the irradiation and so to form calciferol with little or no toxisterol. This effect of the solvent is called the specific solvent effect; it is not clearly understood.

The change from ergosterol to calciferol is purely a photochemical one, involving only a rearrangement of the molecular structure. It is almost unaffected by temperature, though the presence of more than minute quantities of oxygen should be avoided.

The usually accepted series of reactions which occur when ergosterol is irradiated are: ergosterol, lumisterol, tachysterol, calciferol, toxisterol (substance 248), suprasterol I and suprasterol II.

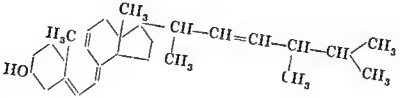
It must be remembered, however, that all these reactions are going on at the same time. It is necessary to stop irradiation when only about half the ergosterol has changed to calciferol, or the continued irradiation will destroy the calciferol which has already been formed. The result is that at the end of the irradiation large amounts of lumisterol and tachysterol with traces of toxisterol are still present. In the Whittier and other processes these drawbacks of making calciferol by irradiation are said to be avoided by activating ergosterol in various ways such as vaporizing it and then exposing it to an electrical discharge [19], but in spite of the often quoted claims for purity made by the manufacturers the resulting products—at least in the Whittier process—owe most of their activity to forms of vitamin D other than calciferol [35] and are no less toxic (p. 578).

Ergosterol has the formula



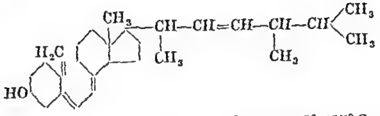
Lumisterol has exactly the same two dimensional formula as ergosterol, but is not precipitated with digitonin, presumably only differing from ergosterol in the relative position of the hydroxyl and methyl groups. It has no antirachitic activity.

In tachysterol



a profound alteration in the molecule has occurred, one of the rings having been ruptured. Tachysterol, so called because of the rapidity with which it reacts chemically, has no antirachitic action.

Calciferol



at 115°–117° C Its maxi
able if kept in a refrigerator
olved in propylene glycol or
m temperatures for at least
three yea ities when heated to about
180° C to be 40 000 I U per mgm
though it han thus [20], esterification
greatly decreases it [21]

The estimation of calciferol in an irradiated solution of ergosterol or in

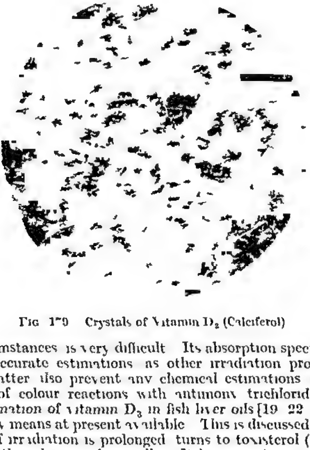


FIG 179 Crystals of Vitamin D_2 (Calciferol)

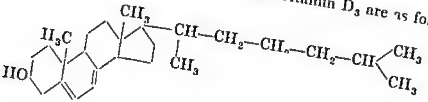
any other circumstances is very difficult Its absorption spectrum cannot be used to give accurate estimations as other irradiation products blur the picture the latter also prevent any chemical estimations so that—apart from the use of colour reactions with antimony trichloride for the very inaccurate estimation of vitamin D_3 in fish liver oils [19 22 23]—biological assay is the only means at present available This is discussed later on p 523

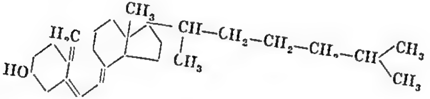
Calciferol if irradiation is prolonged turns to toxisterol (substance 248) about which little is known chemically It has an intense absorption band with its maximum at 248 millimicrons It probably has no antirachitic action though it is toxic With further irradiation it changes to the suprasterols It is formed in the largest amounts when alcohol is the solvent

Vitamin D_3 is formed in the same way as vitamin D_2 only its precursor or provitamin is 7 dehydrocholesterol an animal and not a vegetable sterol
Th hydrogenation of cholesterol
in converted *in vitro* to vitamin
D_3

The absorption spectra both of vitamin D_3 and its provitamin are like the spectra of vitamin D_2 and its provitamin This is the chief reason why for so long vitamin D_2 was thought to be the only vitamin D

The formulae for 7 dehydrocholesterol and vitamin D_3 are as follows





The estimation of vitamin D_3 has the same difficulties as the estimation of vitamin D_2 and so to be accurate has to be biological (p 523) The stability of vitamin D_3 is very similar to that of vitamin D_2 it has been very fully investigated under various conditions and with various solvents by Huber and Barlow [20] The effect of saponification of fish liver oils on the biological activity of the vitamin D_3 which they contain has been investigated by Bailey [21]

Vitamin D_4 is made by irradiating 22 dihydro ergosterol It is an artificial vitamin of no practical interest

There are many other D vitamins of which about twenty have been more or less investigated None as yet appear to be of more than theoretical importance There is however evidence that some fish oils contain further new forms of vitamin D [27] which are especially antirachitic for the turkey [28 29] and are also of clinical value [30] A further complication is that fish liver oils enhance the biological action of vitamin D [31]

UNITS OF VITAMIN D

The International Unit of vitamin D is the vitamin D activity of 0 025 micrograms of the International Standard preparation of crystalline vitamin D_3 which has the following properties

Melting point 87° 89° C (corr)

$[\alpha]_D^{20} = + 110°$ (ethanol)

$E_{1\,cm}^{1\%}$ 265 $m\mu = 490$ (ethanol) corresponding to a molecular extinction coefficient of 18 800

Coward's very interesting paper [32] should be read for her account of how the first standard of reference for any vitamin—originally thought of and used in England—has slowly evolved into the present International Standard For all practical purposes this Standard has the same value as the calciferol one which it replaced so the value of an International Unit in current and earlier work is the same Coward [32] also gives the relative values of other units of vitamin D which were in common use up to 1952

It must be remembered that as children (p 538) and chicks [33] are less sensitive than rats to vitamin D_2 (calciferol) though equally sensitive to the vitamin D_3 of fish liver oils the potency of oils intended for human or avian consumption must be assayed on chicks and not on rats as otherwise any adulteration of the oil by vitamin D_2 will give it too high a value

ESTIMATION OF VITAMIN D

Biological methods have to be used for accurately estimating vitamin D because chemical or spectroscopic methods are only of value for the very crude assay of fish liver oils (p 523) For full details of the biological methods Coward s book [38] should be consulted Four methods are commonly used, the results obtained being accurate to within ± ten per cent

The "Line Test" This is based on the cure of rickets Two identical groups of rats are fed on a rachitic diet until rickets has developed To the

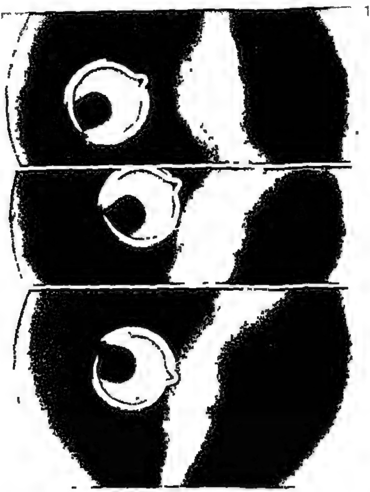


FIG 180 X rays of the tarso metatarsal joints of five weeks old chicks used in Olssen s radiographic method of assay of vitamin D The upper X ray is from a chick receiving natural vitamin D_3 from cod liver oil the middle X ray from a chick receiving the same number of International Units of synthetic vitamin D_2 (calciferol) and the lower X ray is from a chick receiving no vitamin D Note in the top X ray the greater calcification and the narrower tarso metatarsal gap (*See also* Fig 184)

diet of one group is then added a standard preparation of vitamin D of known potency, and to that of the other group the substance to be assayed This curative period requires about ten days The rats are then killed and the distal ends of the radius and ulna in each rat are examined for calcification by splitting the bones and putting them in a solution of silver nitrate The bone which has been deposited during the curative period is thus stained showing as a black line By comparing the "lines" of the two groups of animals the potency of the material being assayed can be deduced Instead of staining with silver nitrate after death sodium alizarinate may be injected

into the animals during life This stains newly deposited bone thus making it easy to see [40] The incisor teeth of rats according to Irving [41] show a more rapid and more delicate response to vitamin D than do the epiphyses

In every fresh assay the standard vitamin D preparation must be used as a control since the response of a colony of rats to the same amount of vitamin D varies greatly at different times The number of rats used depends on the material being investigated If the potency of this is not already roughly known thirty six animals will be required so that the standard and the substance being tested may be compared at three different levels of intake Where large numbers of estimations are being performed the construction of a curve of response as in vitamin A estimations saves time

The X-ray Method This is similar to the line test but the degree of calcification is judged radiographically

The Bone Ash Method This method depends on the prevention of rickets Groups of rats are fed for from four to six weeks with varying levels of the standard preparation and that being tested They are then killed and the bones of the femora ashed using the metaphyses alone being most satis factory [42] Comparison of the weights of the ash from the various groups of animals shows how much calcification has occurred This method is more arduous than the others but gives more accurate results and may be used with chicks [34]

Olsson s Radiographic Technique [34] Here chicks are used instead of rats and the antirachitic potency is gauged by radiographic measurement of the tarso metatarsal gap (Figs 180 184) Baker and Wright [39] state that this method is as accurate as the bone ash method for chicks and has the great advantage that records are easy to keep and that the birds are not sacrificed

Other methods which have not yet been fully confirmed are based on the estimation of plasma phosphatase in chicks [36] and on the external count of the forepaws of rats given radioactive phosphorus [37] It is important in all assays that the animals should eat all their diet especially the phosphorus and calcium and fat When any of these are present in the substance being tested equivalent amounts must be added to the diets of the control animals

PHYSIOLOGY OF VITAMIN D

Vitamin D_2 (calciferol) and vitamin D_3 have broadly the same biological effects and so will be regarded as one in the following pages those differences not already mentioned being fully discussed as they arise especially in regard to their relative toxicity (p 534) and their relative antirachitic value for children (p 538)

The Origin of Vitamin D of Animals Animals gain their vitamin D in two ways by devouring the tissues containing vitamin D of other animals or by the direct action of the sun s rays on the provitamin in or on their own skins Food as a source of vitamin D is generally not important only man in temperate and cold climates and nocturnal animals and birds are driven to use food for a substitute for sun as are stall fed cows and calves reared as they should be in dim byres Such cows [59] or calves [43 44] gain sufficient vitamin D for calving and lactation or good bony development from hay or silage the origin of this vitamin is presumably from sterols activated by the sun during drying in the grass or in fungi on the grass Another rich vegetable source is cacao shells [16]

Vitamin D is sometimes deliberately increased in human food This can lead to some confusion as to what vitamin is really being taken Thus by irradiating a nursing mother or an animal vitamin D_3 is increased in their milk because their own animal sterol has been activated The same result is obtained if the milk itself is irradiated But if a nursing mother or animal is fed on irradiated yeast or any other form of irradiated ergosterol the increase

in the antirachitic power of the milk is due to vitamin D_2 being secreted in it

spread it over their feathers, where it is exposed to the sun and activated It is then probably absorbed by the skin rather than scraped off the feathers by the beak and eaten [45] The removal of the preen gland makes birds more susceptible to rickets, and prevents ultra violet light from having any antirachitic effect unless the feet as well as the plumage are irradiated The fur of animals in a similar way appears to be the place where the vitamin is formed preventing rats from licking their fur destroys the antirachitic effect of irradiation, and owls and young carnivorous birds in captivity have

they are to

and rabbits

method of

rather than

in, the skin Helmer and Jansen [47] found that the fat washed off the bodies of athletes who had been exposed to irradiation before taking violent exercise was antirachitic, while fat from the skin of athletes who had not been irradiated had only a trivial potency Irradiation of this fat made it potent Further, the ultra violet rays of the sun penetrate only some 0 1 mm [48] to 1 2 mm [49] through the skin, so that activation must occur at least close to the surface Possibly the old belief that too much washing makes babies fretful is due to the removal of their vitamin D leading to the fretfulness of rickets After sunbathing it appears possible that swimming is a mistake, the activated fat being washed off the skin before it has had time to be absorbed

The Origin of the Vitamin D of Fish Nothing is definitely known about how fish acquire their vitamin D The plankton in the sea which are the basis of the diet of the small fish, on which the larger fish feed, do not, according to Drummond and Gunther [50], make vitamin D, nor does it seem probable that the sun's rays activate a provitamin in the fish [51] Thus the two ways in which animals acquire vitamins are not open to fish One is driven back to the explanation that fish synthesize vitamin D, though why they do so, or what advantage it is to them to store so much is obscure Apparently the vitamin in many cases is not only vitamin D_3 [75] but also one or more new vitamins of an even higher antirachitic value [27–30]

Assimilation of Vitamin D In birds and furry animals we have already

will cure rickets This, and the fact that vitamin D is active when injected, proves that no essential change has to take place in it during digestion before it can be used by the body Polskin and others [187], from a review of the scant literature find that over ninety per cent of vitamin D is absorbed from concentrated preparations by man, though far less by animals and birds apart from the dog

Bile is essential for the absorption of vitamin D Taylor and his co workers [52] found that dogs with biliary fistulae did not absorb vitamin D when given by mouth unless bile salts were given at the same time Other investigators among whom is Heymans [53], have confirmed these results by very similar experiments on rats or dogs No analogous work has been done on man, but there seems no reason to doubt that similar results would be obtained

orption since fat free tablets of

poisoning (p 578), but vitamin

through the intestinal wall as

impaired fat absorption in man may cause rickets (p 561) or osteomalacia

side it degenerates being then invaded by capillaries and osteoblasts or bone forming cells Bone salts are then laid down in the degenerating cartilage but the formation of new cartilage keeps pace with its degeneration so that growth in length occurs by the invading bone on the diaphyseal side of the cartilage always chasing the retreating new cartilage on the epiphyseal sid Growth ceases when no more cartilage is formed so that the diaphyseal bor reaches and fuses with the epiphysis The thickness of the bone is increase by osteoblastic activity underneath the periosteum

FIG 182 Foetal rickets in a Chinese baby A section of the distal end of the ulna stained with silver nitrate Note the expanded cartilage and osteoid tissue and the ragged diaphyseal end of the ulna (*See also* Figs 186 and 189)

In rickets the band of cartilage widens because it goes on growing but ceases to degenerate A large number of osteoblasts and capillaries appear between it and the diaphysis forming a mass of osteoid tissue that is the organic part of bone without the inorganic No calcification takes place since degeneration has ceased in the cartilage The large amount of osteoid tissue and expanded cartilage causes the classical swellings for instance of beaded ribs and enlarged ankles and wrists Under the periosteum of the bone calcification stops though there is an increase in vascularity and osteoblasts forming osteoid tissue The finer structure of the bone and the trabeculae of the marrow cavity is affected because decalcification also occurs Clinically this results in weakening of the bones with bending and fractures

The X ray picture in severe rickets shows these changes In normal growing bone the end of the diaphysis is smooth and there is a clear space between it and the epiphysis But in rickets the end of the diaphysis is uneven and ragged because the degeneration of the cartilage and subsequent calcification has stopped unevenly A faint curved shadow along the edge of the cartilage may sometimes be seen extending towards the epiphysis this is the faintly calcified perichondrium round the swelling caused by the uncalcified mass of osteoid tissue and overgrown cartilage The shaft of the bone also shows rarefaction with a general coarsening of the structure while the outline is irregular and blurred by the shadowless osteoid tissue under the periosteum (see X rays in Figs 183 189 and 194)

When healing starts under the infl
(which can be shown histol
of degeneration of the
lose their glycogen [99]
and the laying down of

capillary penetration
The result is that a line of preparatory

calcification is formed close to the ragged edge of the diaphysis which can be seen on X-ray examination, forming the "line test" for healing rickets. Bone salts are also laid down in the osteoid tissue at the ends of the bones and along the shafts, so that in both places denser shadows appear in an X ray (Fig 196)

In adult animals deprived of vitamin D the picture must be modified because growth is no longer taking place The changes have to be limited to the decalcification of the bones, and the formation of osteoid tissue under the periosteum, with a resulting weakness giving clinically the picture of

Fig

osteoporosis or in severe deficiencies, osteomalacia The rôle of vitamin D in the calcification of the teeth is discussed on p 570

The other changes which occur in rickets are

(*a*) The amounts of calcium and inorganic phosphorus in the serum are altered In experimental rickets these elements largely mirror their content in the food, so that rickets can be produced with widely varying amounts and proportions of these two present in the serum In human rickets (p 559) the phosphorus is generally low, the calcium normal, but the latter may be grossly reduced In osteomalacia the calcium is often reduced to tetanic levels, but it may remain nearly normal with very low phosphorus levels (p 568)

(*b*) The plasma alkaline phosphatase (p 558) is constantly raised in rickets

and, to a less degree, in osteomalacia This rise, however, can be prevented in animals by depleting the diet of manganese [88] The origin of this phosphatase is the bones [36]

(c) The calcium and phosphorus of the fæces is increased, of the urine decreased

(d) The skeletal muscles lose their tone, and the ligaments become lax

(e) *The smooth muscle of the gut also loses its tone* This is important, as Yoder [89] reports that it may increase the time food takes to pass along the gut by as much as twenty six per cent He points out that this relative stagnation of the food means that chemical changes take place in the gut as the result of decomposition of the food, and not as the direct result of lack of vitamin D He believes the alkalinity of the fæces in rickets is due to this decomposition, since the reaction can be restored to normal either by vitamin D or purgatives

The Relation of Vitamin D to Growth. Stimulation of growth, as apart from the prevention of rickets, is an important function of vitamin D Such clinical observers as Gardiner Hill [90] and Still [91] state that a deficiency of vitamin D decreases growth quite apart from loss of stature due to rachitic deformities (Figs 178, 188) This has been confirmed in rats [98] and lambs [96] while Mellanby [74] states that in puppies vitamin D above the amount needed to prevent rickets improves the architecture of the bones Large scale investigations on children also show that amounts of vitamin D above those necessary to prevent rickets lead to increased growth [92] Norman's important work [93] on the difference in height between children of the richer and poorer classes also would suggest that vitamin D is related to growth since it is one of the most deficient vitamins in the diet of the poor.

Whether it is an advantage to the individual to be taller is debatable That children and experimental animals grow more on large amounts of vitamin D does not mean that such doses of vitamin D are the correct ones It may equally well be held that large amounts of vitamin D stimulate excessive growth Jeans and Stearns [94] found that when vitamin D is given in still larger doses growth is impaired, even though toxic symptoms are absent Analogous results are reported by Speidel and Stearns [95], since giving infants 300 to 400 I U. of vitamin D daily caused an earlier eruption of the first deciduous incisors than did larger or smaller doses The body appears to have no power to regulate the amount of vitamin D it absorbs in the food, whether it can regulate that formed in the skin by irradiation is unknown But as the latter appears to be the natural way of acquiring vitamin D experiments suggesting the value of a high consumption of the vitamin should only be accepted if the results are the same as are given by irradiation Any results which appear to suggest feeding is better than irradiation may mean that they are not truly better, but are the effect of abnormal stimulation If growth is taken as the criterion for measuring the correct dose of vitamin D we should know what is optimum growth We do not

The Relation of Vitamin D to the Endocrine Glands. *The Parathyroids* [104] The parathyroids being so intimately connected with the metabolism of calcium and phosphorus must have some direct or indirect relationship to vitamin D It has even been suggested that vitamin D only acts through stimulating these glands This cannot be so, since the action of vitamin D and parathormone is *quite different* With small doses the former keeps the serum calcium and inorganic phosphorus normal, and caused a positive balance of both in the body The latter raises the blood calcium after decreasing the inorganic phosphorus by diuresis, and causes a negative balance of both Excessive amounts of the hormone and vitamin have different actions on bone the former causes decalcification and replacement with fibrous tissue and giant cells, while the latter causes dissolution of the trabeculæ with no fibrous replacement

Clinically the difference is important, since rickets is cured by vitamin D but made worse by parathormone. Further, in parathyroid tetany the beneficial effect of parathormone wears off, but the effect of vitamin D does not [104, 112]

In rickets and osteomalacia the ged and over active vitamin D causes 13] In dogs [130] hypervitaminosis D d

Possibly vitamin D should be considered as regulating the metabolism of phosphorus and the parathyroids as regulating phosphorus excretion Sometimes the hormone acts with and sometimes against vitamin D according to the needs of the body

The Thyroid and Pituitary The basal metabolic rate is decreased in rickets, but this is explained by Nicolaysen [105] as being due to decreased activity, since he found in narcotized rats that the carbon dioxide output was the same whether rickets was present or absent

Vitamin D in doses on the threshold of toxicity raises the basal metabolic rate by stimulating the thyroid *via* the thyrotrophic mechanism of the pituitary [105, 107], it seems doubtful if this can have any clinical importance. With toxic doses the thyroid appears to be over-active and there is an increase in the eosinophils of the pituitary [108] Vitamin D_2 and calcium chloride, though not calcium carbonate, increase the size but not the iodine content of the thyroid glands of rats on a slightly goitrogenic diet [114] The darkening of the feathers of rachitic chicks [100] may be due to some endocrine disturbance

The Thymus and Pancreas There is some evidence that the removal of the thymus in animals increases the severity of rickets and decreases the effect of vitamin D [109, 110] Rickets in rats is said to give some protection against alloxan to the beta cells of the pancreas [101]

The Sex Glands In osteomalacia the regularity of the menses and the disastrous recurrent pregnancies suggest that lack of vitamin D has no effect on reproduction in woman It has been reported by Reed and his collaborators [111] that huge doses of vitamin D have occasionally increased sexual capacity and libido (even to an inconvenient extent) and also increased the regularity of the menses Toxic doses decrease the size of the prostate and testes [130] and spermatogenesis [108] in the dog, but in the rat have no effect on this or ovulation [106], though lack of vitamin D in the latter animal causes dicestrus [115] In mice the endosteal bone formation caused by œstrogens is not dependent on an adequate supply of vitamin D [116]

The Relation of Vitamin D to the Blood. Gray and Ivy [117], and McNealy and others [118] have shown that vitamin D prevents the hæmorrhagic tendency which is so common and dangerous in patients with jaundice and hepatic insufficiency (p 576) Why the vitamin has this effect is obscure

time of sedimentation rate [111] In dogs large doses decrease capillary permeability [121] The thrombocyte count in rats is said to be increased by large doses of vitamin D [119] and rachitic rats absorb iron and form hæmoglobin slightly less well than animals receiving vitamin D, especially when this is from fish liver oils [122]

The Relation of Vitamin D to Infection During the last century in England cod liver oil was greatly prized for its value in tuberculosis [123], which has been amply confirmed, at least as regards vitamin D in the treatment of some forms of tuberculosis, in recent years (p 572) This added to the commonness of catarrhal infections in rickets, and the clinical value of sunshine and ultra-violet light in improving the general condition of consumptive and convalescent patients, has led to a widely held belief that vitamin D increases the resistance of the body to infections But the results of animal

experiments in which the formation of antibodies, etc, have been studied are inc ... *of vitamin D* is the ... ency It must be rem ... used clinically the oil and vitamin A of the former and the general stimulation from the latter are valuable quite apart from any action of vitamin D itself Reed and his collaborators [111] in 1939 summed up their excellent review of the literature on vitamin D and infections by saying " there is no proof of a specific effect in any type of infection with the possible exception of tuberculosis " More recently Tomey [124], using monkeys, has shown that rickets reduces resistance to the virus of poliomyelitis when this is injected into the wall of the gut or into the suprarenals Rachitic nerves ground up with the virus absorb it while normal nerves do not Weaver and others [125], however, having inoculated cotton rats with the virus in every possible manner, could not confirm that lack of vitamin D had any effect on susceptibility or on the development of resistance Tomey's findings are probably of no clinical importance since the seasonal incidence of poliomyelitis is not that of rickets, nor has poliomyelitis ever been found to select the rachitic and poorly fed Young mice are also reported to be more susceptible to swine influenza virus when deprived of vitamin D [126] The clinical results of treating infections with vitamin D have been too disappointing to consider

Effects of Excessive Vitamin D All forms and preparations of natural and synthetic vitamin D are toxic when given in sufficiently large amounts, though the belief still lingers that pure vitamin D itself is not toxic because the first workers with massive doses of the vitamin ascribed its toxicity wholly to the toxic impurities, such as toxisterol, which were present in the very impure irradiated ergosterol which they used Actually pure or highly purified preparations of vitamin D_2 and concentrated fish liver oils are toxic for the rat [127 128] and the dog [129, 130 131] and man (p 578) Vitamin D_3 is definitely more toxic than vitamin D_2 for rats [127] and dogs [131] and so it is reasonable to suppose it would be so for man, though no work has been done on this subject Toxicity is reduced in animals by very large amounts of vitamin A [127, 131, 132], this has not been confirmed in clinical work [111], though aneurine or yeast are often stated to be of value [111 133]

Excessive doses of vitamin D mobilize the phosphorus and calcium from the tissues of the body, thus broadly having an opposite effect to normal doses The soft tissues tend to become calcified the bones to be rarefied—in growing bone the cartilage acts as a soft tissue The soft tissues most affected are the tubules of the kidneys [129] the media of the arterioles of the kidney [134] and the media of the large blood vessels, though the bronchi, lungs, heart and stomach are also involved [130] The aorta, for instance, in animals kept for some time on sublethal doses looks exactly like that found in old atheromatous men This metastatic calcification takes place after the tissues have already been damaged by the excess of vitamin D, it is thus a secondary change and not the primary one [43] Casts composed of calcium salts are often present in the urine if this is not too acid If the toxic doses of vitamin D are stopped the calcareous deposits may largely disappear [135] The serum phosphorus and calcium tend to be grossly raised but not always—so that a raised blood calcium does not of necessity give a warning that the amount of vitamin being taken is toxic [111] A diet rich in bone salts increases the metastatic calcification, but a diet deficient in salts does not decrease the fundamental damage to the tissues Opper [136] states that metastatic calcification is increased in rats when their renal function is impaired either by a diet deficient in vitamin A (p 47) or by ... In man [111] the blood pressure is not affected but in ...

diarrhoea and loss of weight and may die in a few days. In dogs [130] there is atrophy of the testes and the prostate while the parathyroids are smaller than normal with contracted nuclei (see also p 532). The clinical picture and post mortem findings in human cases poisoned by vitamin D are described on p 47

Fundamental Nature of the Action of Vitamin D The effects of vitamin D are best explained though it must be adr sidering that its action is primarily on phosphoru e whole body [138] essentially activating the Thus vitamin D not only mobilizes phosphorus from the tissues so aiding its combination with calcium [142] by converting organic phosphorus into an inorganic form [139] but it also has an effect on the metabolism of phosphorus during muscular work [143]

Nicolaysen [82 144] and Harrison and Harrison [147] have shown by most careful work on rats that calcium absorption is increased by vitamin D but that this effect is largely dependent on the needs of the body for calcium especially in the young [82 147] in the adult both vitamin D and the needs of the body for calcium have much less effect on absorption which has been confirmed for women [148]. Nicolaysen [144] did not find that the absorption of phosphorus from isolated loops of the intestine was different in rachitic and normal animals though Laszt [87] using the same technique reports that the absorption of phosphorus is decreased in rickets. But whether or no vitamin D directly affects phosphorus absorption it is probable that its effect on calcium absorption is really a secondary effect due to it rendering the phosphorus in the cells of the gut wall sufficiently labile for the calcium to combine with it and so be absorbed [149]. Congruous with this theory is the belief of Migicovsky and Emslie [150] who from experiments on chicks hold that the sole action of vitamin D is to prevent bone being dissolved away into the blood and so decreasing the vacuum for calcium in the cells of the gut wall. Greenberg's work with rats [151] can also be interpreted in a similar manner though he himself adds to a direct enhancing action on the mineralization of bone a further direct enhancing action on the absorption of calcium

Accepting the above theory of the action of vitamin D calcification is started by the conversion of the organic phosphorus of the serum and bone to inorganic phosphorus. This so raises the concentration of the latter that it can combine with the calcium of the serum to form insoluble calcium phosphate aided in the neighbourhood of growing bone by the action of Robison's bone phosphatase on hexose phosphoric esters [145]

It must be stressed that bone salts are not laid down because an increase in inorganic phosphorus in the serum automatically causes precipitation of calcium phosphate they can only be laid down by the action of living cells [140] when the concentrations of salts bathing these are sufficiently high

Rachitic bone and cartilage have no fundamental inability to lay down bone salts since they do so if they are placed in normal serum or suitable salt solutions [140]. In living rachitic animals the injection of inorganic phosphorus and calcium also causes calcification [141] since the salts are artificially put into the fluid bathing the cells in a form which is only produced naturally by the action of vitamin D

The poor tone of the skeletal and visceral muscles in rickets is possibly due to the effect vitamin D has on phosphorus metabolism in muscular work [143]

The damage done by excessive amounts of vitamin D (p 534) may be due to an excessive mobilization and conversion of organic phosphorus to inorganic phosphorus not only in the soft tissues but also in the bones a mobilization which is injurious in itself only secondarily leading to metastatic calcification [129] when enough calcium is present to combine with the liberated phosphorus

Another theory of the toxic action of vitamin D is that it is due to an impurity formed during irradiation, such as toxisterol (p 520) In favour of this is that unit for unit the least purified products of irradiated ergosterol are the most toxic [146] Toxic symptoms again often occur only after some intestinal upset which might have led to decomposition of vitamin D in the gut before absorption with the production of injurious substances [111] Aneurine has a protective action against overdosage of vitamin D, which may be explained by the value of the former for the proper functioning of the gut, which would decrease any tendency to intestinal putrefaction [111] As however, very pure preparations of calciferol have a toxic action when injected one must also postulate that the cells of the body cannot completely destroy huge doses of vitamin D, but form from it some toxic substance like toxisterol Here again adequate aneurine should aid the cells in the complete destruction of vitamin D. This theory does not run counter to the theory that excessive vitamin D upsets phosphorus metabolism, but only tries to explain further the underlying mechanism

SOURCES OF VITAMIN D AVAILABLE TO MAN

Sunlight and Ultra-violet Light. Sunlight acting directly on the body should be the way in which vitamin D is obtained But in northern climates and large cities clothes, window glass, smoke, and clouds cut off most of the active rays Even so by letting infants and children be exposed as far as possible out of doors and by opening nursery windows much benefit is gained In one hospital in the heart of London it is found that infants always thrive better if they are out on a roofed verandah whatever the weather This raises the point that the action of sunlight and fresh air is not only due to the formation of vitamin D but also to a general tonic effect on the body, the fluctuating temperatures on the skin, for instance, cause a stimulation of the thyroid-suprarenal mechanism

Sunlight in excess is a powerful, though delayed, poison which may cause

used with care, especially for infants The eyes and head must be shaded by a wide brimmed hat As some children are more sensitive than others a ten-minute exposure of the arms and legs is enough for the first few days The duration and amount of the body exposed may be gradually increased, but complete exposure of infants for more than half an hour twice a day is excessive When the sunlight is very intense sun bathing is best in the cooler parts of the day—early morning and evening With adequate sunlight the amount of exposure recommended above will cure rickets as rapidly as the doses of vitamin D commonly used For adults sun bathing is of value but the prolonged sudden exposure of city people on short holidays is harmful

Artificial sunlight given either by the carbon arc or mercury vapour quartz lamp, is an excellent substitute for sunlight, having the same value in the prevention and cure of rickets and also the same tonic effect Hill and Laurie [48] found in a carefully controlled experiment that children benefited more from irradiation than cod liver oil, their weight appetite and sleep being improved the number of colds decreased and nervousness lessened But irradiation needs to be used with caution Both the duration of exposure and the distance of the bather from the lamp must be carefully supervised It is essential that the eyes are always protected with coloured glasses Both adults and children may be unduly sensitive to irradiation, especially those who are fair The first exposure should be of short duration to make certain there is no intolerance A short transitory erythema should be produced, but

any other symptoms suggest the exposure is too prolonged. We have seen [illegible]

instructions for their use cannot be given here, but must be obtained from a therapist or the makers.

Food. The dietetic sources of vitamin D can be divided into (*a*) normal foods, (*b*) fortified foods, and (*c*) concentrated natural and artificial preparations.

Normal Foods. Vitamin D is poorly represented in food. The only good sources are dairy produce, fatty fish, and dripping. Green vegetables, in spite of all belief to the contrary, do not contain vitamin D.

The value of all dairy produce depends on the diet and exposure to sunlight of the hens and cows. Hens' eggs contain nearly three times as much vitamin D in the summer as in the winter. The eggs of commerce produced in abnormal numbers by birds under the loathsomely cruel battery system may be almost valueless from the point of view of vitamins D and A

English butter may only contain 3 I.U of vitamin D in 1 ounce in the winter, though in the summer there may be 130 I U These figures are extreme values: butter from cows which are kept outdoors in the winter should never have such a low winter value. The importance of butter is shown by Friend's [152] report on the health of the boys at Christ's Hospital School In 1918 the War made it necessary to give the boys less milk and unvitaminized margarine instead of butter The average fracture rate till then had been 0·75 per cent During the next four years the average fracture rate rose to 1 78 per cent., in 1922 even being as high as 2 38 per cent Yet in 1922 all the War rationing had disappeared, the diet being as good as it was before 1918. The only difference was that margarine was still being eaten instead of butter. When butter was again given the fracture rate sank to normal. The explanation offered is that the vitamin D of the butter saved the boys from mild rickets or osteoporosis. But the amount of vitamin D in butter is small, so that it seems possible that the fat of the butter itself or some as yet unidentified factor in butter (p 680) or the form in which it contains vitamin D was the important factor. In favour of this is the observation by Boer [155] that whole butter has eight times the antirachitic value of its unsaponifiable fraction, which suggests that butter, like milk, increases the potency of the vitamin D it contains.

Milk is an extremely valuable source of vitamin D This is not due to the actual amount of vitamin D present, which is relatively small, but because its antirachitic value is greatly enhanced when it is given in milk. Thus Hess and Lewis [154, 156] reported that unit for unit "yeast" milk (p. 538) was five to ten times as antirachitic as calciferol dissolved in oil. Similar observa- [illegible] diluter concentrations nor to finer dispersion, but is the result of vitamin D forming a compound with the lactalbumin of the milk. This compound is more effective than vitamin D alone. It must also be remembered that the lactose of milk is of value in aiding calcification; cane sugar and starch have not this property [86]. Human milk is discussed on p. 543.

Sir John Russell [160] has pointed out that milk at present need not conform to any [illegible] ion should be introduced to [illegible] vitamins A and D, since th[illegible] for these vitamins. Legislation of this type has already been introduced in Finland [161]

Fatty fish are a valuable and relatively cheap source of vitamin D. Of special value are fresh or tinned herring, bloaters, pilchards, kippers, sardines,

salmon and eels In 1939 the day's requirements of vitamin D could be bought as herring for three halfpence, as tinned salmon for fourpence, and as eggs for tenpence [162] but, of course, by 1952 only the former remain freely available Fish are also a good source of iodine for man, and of calcium and phosphorus [163, 164] if their bones are eaten, as they may be in the case of sardines, sprats, and tinned herring and salmon The bones of salmon have been found to have a high antirachitic value for animals [163] It used to be said in the days of plentiful cheap food that tinned salmon was the Londoner's sunlight, but even now, in the days of dearth the still plentiful herring does not hold this title, even though the English Herring Fleet could land far more herring than are eaten at home [165] In 1937 the average individual daily intake of vitamin D from herring, fresh or canned, was only 35 I U [166] though one fresh herring provides the day's needs of vitamin D

Butcher's dripping bought in July in England is stated by Henry and others [167] to be as good a source of vitamin D as "summer butter," and, of course it has a higher energy value than margarine

Lindsay and Mottram [162] have explained how in the preparation of food additional vitamin D can be incorporated by the substitution of cod liver oil for olive oil or butter in such things as white sauce, mayonnaise, and batter when these are for fish dishes where the faint taste of the oil is masked by that of the fish

Fortified Foods Cowell [168] in 1925 first irradiated food as a means of increasing its vitamin D In spite of the fact that this pioneer work was English, its commercial applications have been largely ignored in England while widely used in the United States

Vitaminized margarine is practically the only food in England to which vitamin D—as vitamin D_2—is added, all margarine must contain, by law, 90 I U per ounce with the exception of that doled out by the Ministry of Food to cake shops, etc, which contains none [171] In any case margarine can never be a true substitute for butter, the only important point is to prevent the public from believing it is But since margarine instead of butter is an economic necessity in many families, high praise is due to the manufacturers whose own private work on vitaminization has enabled the Government to insist on it officially

Milk is widely reinforced, especially in America This can be done by (1) irradiating the cow, (2) irradiating the milk, (3) giving the cow vitamin D in her food, (4) adding vitamin D to the milk By the first two methods vitamin D_3 is increased in the milk, as it is if fish liver oil is given in her diet, or is added later to the milk But in both the latter cases fish oils may give a fishy taste to the milk Vitamin D_2 is free from this objection and can be added directly to the milk or fed to the cow as irradiated yeast, giving "yeast milk" Irradiated milk generally contains about 135 I U per quart, while "yeast milk" and milk to which vitamin D is added generally contains 400 I U per quart The great advantages of giving vitamin D in milk are discussed on p 537

Cheese, or at least a substance which those with no palate could be deluded into thinking was cheese, can now be fortified with fish liver oils [242]

Concentrated Preparations of Natural and Artificial Vitamin D Natural vitamin D_3 from the work discussed below, has about twice the potency of artificial vitamin D_2 This is important when deciding what concentrated preparation shall be prescribed those widely available include good cod liver oil and concentrated fish liver oils, both containing vitamin D_3 solutions of vitamin D_2 in vegetable oils, tablets of vitamin D_2, capsules of concentrated oils which may contain either vitamin, the Ministry of Food Cod liver Oil Compound, containing a mixture of both vitamins in unknown proportions [171], and foods like infants' cereals and sugars which often have added vitamin D_2

The superiority of vitamin D_3 over vitamin D_2 would appear to be

definitely proved Thus May and Wygant [157] in a study of four hundred and fifty seven infants decided that while 200 to 500 I U of vitamin D given daily as cod liver oil were sufficient to prevent rickets 400 to 800 I U of calciferol in oil were necessary to obtain the same effect Hess and his collaborators [154 156] from a study of sixty infants also give approximately the same figures which are strongly supported again by Houet [172] who judged the relative efficacy of the two vitamins by their effect on mineral absorption—an investigation doubly important because it used another yardstick to those previously employed by other workers yet obtained the same result Jeans [173] concludes that vitamin D of animal source appears to be more potent for the human being than the vitamin D of vegetable source (calciferol) Brockman Rietschel and others [174 175] state that vitamin D_3 is superior to calciferol when only one massive dose is given to protect children against rickets throughout the winter Gerstenberger [176] comes to the same conclusion from experiments with monkeys McBeath and Zucker [177] found the synthetic vitamin less valuable than the natural for increasing the immunity of children to dental decay (p 570)

Most investigations [158 159] which have purported to show that the two vitamins are of equal value are fallacious because doses were used which were

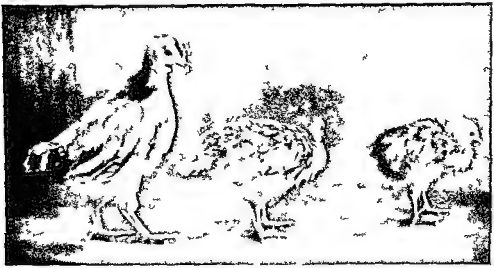


FIG 184 Five weeks old chicks The bird on the left received natural vitamin D_3 from cod liver oil the bird in the centre received the same number of International Units of synthetic vitamin D_2 (calciferol) and the bird on the right received no vitamin D The average weight and mortality of the ten birds in each group were with the natural vitamin 399 grams and no deaths with the synthetic vitamin 346 grams and five deaths with no vitamin 259 grams and six deaths (*See also* Fig 180)

so large that the prevention or cure of rickets was inevitable by either vitamin and so of course no difference between them could be shown An apparent exception is the work of Glaser and others [178] who gave small doses to premature infants and judged the effect by phosphatase levels and X rays But even here too small a number of infants was investigated and too small a proportion developed rickets for any definite conclusion to be drawn

Further advantages of vitamin D_3 are that it is less toxic at least for animals (p 534) that given as it generally is in cod liver oil may enhance its effect (p 522) that the greater antirachitic value of the natural vitamin for chickens increases over that of calciferol as the doses of the two are increased [184] This suggests that for human beings the superiority of the

natural vitamin might be enhanced at intakes above the minimum for preventing rickets An analogy is found in the greater value of vitamin A than its provitamin (carotene) at levels of intake above the minimum for good growth

Cod liver oil is the best concentrated source of vitamin D for supplementing the diet of both children and adults Its advantages are (1) It provides the natural vitamin D_3 (2) It provides vitamin A (3) It is nutritious (4) A toxic dose of vitamin D cannot be given as ordinary cod liver oil owing to its bulk This was emphasized by Thatcher [179] in his account of a child who died from overdosage of a concentrated oil (5) The work of Jeans and Stearns [80] suggests that concentrations of vitamin D higher than those found in ordinary cod liver oil are not well absorbed (6) Cod liver oil is of value apart from its vitamins, containing, for instance, traces of iodine, and a high proportion of unsaturated fatty acids akin to those essential unsaturated fatty acids which sometimes are referred to as vitamin F (p 671) (7) The cod liver oil requirements of England can be supplied by the English fishing fleet [165]

The traditional objection to cod liver oil is its taste But poor children attending out patient departments often ask for it, and one has the impression that richer children largely dislike it because their mothers tell them it is unpleasant

Concentrated fish liver oils can be used in those rare cases where ordinary cod liver oil cannot provide enough vitamin D, or where the taste is intolerable or the fat injurious as in cœliac disease

Manufacturers of concentrated preparations of vitamin D should state on the bottle that there is a risk of overdosage if more is taken than is prescribed We have seen one patient who drank a preparation of calciferol, merely because he liked it, in such quantities that he was showing the early signs of hypervitaminosis D (p 578)

Effects of Cookery, Storage, Canning, Freezing and Drying on Vitamin D Domestic cookery causes no loss of vitamin D, since it is not soluble in water nor easily destroyed by heat Milk loses none by boiling or by pasteurization Tinned and dried milks have the same vitamin D content as fresh milk, but the analyses of Bacharach and others [166] suggest that there is considerable loss when fish are canned or cured, since the average value of vitamin D in fifteen samples of fresh herring caught throughout the year was found to be 250 I U per ounce, and in five samples of canned herring only 50 I U per ounce As however, the fresh and tinned herring did not come from the same catch these differences may not be entirely due to the canning Dried eggs lose a considerable amount of vitamins A and D when prepared by band drying but none by spray drying [170] The value of butter is not impaired by its storage during transport over long distances, such as those between New Zealand and England [181]

AMOUNT OF VITAMIN D IN FOODS

This table has been chiefly compiled from the tables of Fixsen and Roscoe [181] and McCance and Widdowson [183]

The values of most of the fish, apart from salmon, lamprey and lampern, are only approximate, since research has been directed to the amount of vitamin D in the body oil of fish To make this work of value in human diets it has been assumed that the fat in the edible parts of the fish is body oil and so since the amount of fat is known, the amount of vitamin D has been deduced

Food				International Units of Vitamin D —probably D_3 in 100 grams or roughly 3½ ounces
Butter				
	Danish	November–January		30–8
		February–April		8–14
		May–July		36–54
		September–October		44–15
	Dutch	General		21–48
	English	November–March		10
		April–June		15–40
		July–August		55–97
		September–October		40–15
	New Zealand	General		30–57
	Scotch	November–January		30–8
		February–April		8–22
		May–July		60–99
		August–October		50–20
Dripping				
	English butcher's	July		33–44
Cacao Butter				30 000 as D_2
Cheese				Present ? amount
Cream				
	English cow's			50
Eggs				
	Hen's	Whole		70
		Yolks only	Summer	390
			Winter	140
		Whites only		0
		Whole dried		220 (p 540)
	Duck's and other egg			? as in hen's eggs
Fish				
	Cod			52
	Cod's roe			65–80
	Cod's liver (fresh or canned)			6 000
	Eels			474
	Herring	Canned with Tomato		52–322
		Canned without Tomato		11–420
		Fresh English	August	294–875
			September	930–1 676
			November	735
			December	591–1 270
			March	521
			July	686
	Kippers			590–744
	Lampern			120
	Lamprey	Sea		110–400
		River		33
	Mackerel			304–405
	Oysters			5
	Salmon	Canned	Chum and Chinook	200–300
			Pink	600–700
			Red	800
	Sardine			1 800
	Shrimp flesh			147
	Turbot			27

Food	International Units of Vitamin D —probably D_3—in 100 grams or roughly 3½ ounces
Fish Liver Oils	
Cod	8 100–30 000
Cod minimum allowed in English and American medicinal cod liver oil	8 500
Cod Liver Oil Compound (Ministry of Food)	20 000 as D_2 and D_3
Halibut	20 000–400 000
Tunny (various kinds)	1 600 000–25 000 000
Fungi	
Edible	83–125 as D_2
Mushrooms Grown in dark	21 as D_2
Grown in light	63 as D_2
Green Vegetables	0
Hay	32–200 as ? D_2
Liver	
English Calf	0
American Calf	10
Lamb	20
Ox	40–50
Pig	10–50
Margarine	
Vitaminized	315 as D_2
Unvitaminized Vegetable	0
Milk	
Human (see p 543)	—
Cow's Summer	2·4–3·8
Winter	0·3–1·7
Colostrum Summer	8
Winter	4
Irradiated milk	Generally standardized at 12
Yeast milk	Generally standardized at 35 as D_2
Fortified milk	Generally standardized at 35 as D_2 or D_3
Sow's	4·5
Silage	56–87 as ? D_2
Vegetable Oils	
Arachis or Pea Nut	0
Olive	0
Whale Oil	
Blubber	75
Liver	75

HUMAN REQUIRE[...] OF VITAMIN D

The dietetic requirements for vi[...] [...]iven in the following pages are for the inhabitants of temperate cli[...] [...] are receiving little irradiation from the su[...] [...]g the summer [...] the [...] less vitamin D need be ta[...] [...]d It is assu[...] diet [...]ly abnormal

given especially to infants

are probably excessive especially for premature infants

Premature infants (under $5\frac{1}{2}$ lb at birth)	1 400
Full term infants	700
Children 5 14 years	500
Puberty and adolescence	300 600
Adults male and female	300 600 ?
Pregnancy and lactation	700
Old age	300 ?

The U S A National Research Council in 1948 recommended 400 I U daily for all infants children adolescents and pregnant and nursing mothers while for night workers the elderly and nuns a small amount is desirable The need of extra vitamin D for normal adults seems to be minimum

Breast fed full term babies often develop rickets Premature babies and those which grow very rapidly generally do so unless they are given extra vitamin D If surprise is felt that breast milk should ever be inadequate it must be remembered—quite apart from the mother s diet being often deficient in the vitamin—that children should gain vitamin D from the sun light on their bodies their milk probably only supplements their sunlight rather than the other way round Cow s milk or other artificial foods always require additional vitamin D The problem is to decide how much vitamin D infants require not only to avoid rickets but also to give the best rate of growth (p 532)

Human milk is reported by some workers to be a parsimoniously adequate source of vitamin D for the suckling while others declare it to contain little or none Thus Dr

68 *I U* per quart

in towns during t

was little more th

200 I U daily were taken by the nursing mother larger but still physiological additions caused no further improvement in the milk In contrast to these figures Polskin and his collaborators [187] in the U S A report that during the first seven to nine days of lactation eighteen of twenty one women

while the other

the latter half

en when given

as single weekly doses of 32 000 I U amounting in all to such grossly unphysiological figures as 256 000 to 480 000 I U the content of the milk never rose above 62 I U Taken during lactation however 40 000 I U daily raised the value to between 125 and 504 I U per quart—which is presumably merely an overflow and not a secretion More in keeping with these U S A figures are the further English figures of Kon and Mawson [188] who analysed the pooled milks from a large number of women the samples being representative of the whole twenty four hours thus avoiding fallacies introduced by variations in fat secretion etc at different times of the day [188] The average value per quart was 6 to 13 I U with possibly an increase in later lactation and during the summer

Milk even human milk being so frequently an inadequate source of

vitamin D some extra vitamin must be given Jeans and Stearns [180] found that for three months old infants 70 to 100 I U of vitamin D_3 in cow s milk gave protection against rickets but the maximum amount of growth—if this is really

while doses of 1

dentition [95]

Barnes [30] also considered that 600 I U provided by fish liver oils from whatever fish is ample since forty eight infants with rickets were cured as rapidly with 600 as with 900 or 2 400 I U cure being assessed by blood phosphatase estimations Corner [100] in her very careful investigations on town children living at home found that a supplement of 400 I U daily had no effect on the incidence of rickets which she considered meant either that this amount is inadequate or that it cannot overcome the other contributory causes of rickets among poor town children this is largely confirmed by Krestin [191] who reported that the free distribution of oil containing 400 I U per daily dose to pregnant and nursing mothers and to infants had no beneficial effect But it must be remembered that both these observers were dealing with the poor and so could not be certain the oil was being taken regularly This is a strong argument in favour of providing free cod liver oil with a higher content of vitamin D than would in theory be necessary in order that when a dose is forgotten it is compensated for by the next dose

From the clinical point of view therefore breast fed infants should be given two small teaspoonfuls of an ordinary cod liver oil a day this will contain about 500 I U For artificially fed infants another teaspoonful should be given The oil is started about a month after birth in 5 drop doses thrice daily dropped on the tongue and increased slowly to the full quantity in about three months

Premature infants suffer from two drawbacks—they grow very fast and they have not stored the b

in the last weeks of fœt

minerals can seldom be ab

is inevitable but this can

doses of vitamin D are gi

taken in 5 drop doses thrice daily and rapidly increased if it does not cause indigestion The oil can be dropped on the tongue More concentrated preparations can be tried but they are probably less well absorbed (p 540) Not less than 600 I U appears to be the dose which should be aimed at as soon as possible [30 192 193 194] the 1 400 I U advised by the British

pear to be large enough to check growth and

oil with the milk in the feeding bottle is

float to the top and stick to the sides of the

bottle

Massive single doses of vitamin D are sometimes given orally or by injection to infants to confer prolonged protection against rickets both on the Continent and in America This form of treatment should never be introduced into English medicine The advocates of the single massive doses claim that it is safe that the time of the mother is not wasted by frequent visits to baby clinics to obtain cod liver oil she cannot afford to buy and that the infant of the most feckless mother can be protected for several months against rickets though he is only brought to a clinic once Such arguments granting the dubious assumption discussed below that one huge dose of vitamin D is not toxic are only valid in backward countries in which unlike England there are few doctors and the poor are not educated to bring their infants regularly to clinics In England every effort should be made not to curtail the number of visits to infant welfare clinics but to increase them Rickets is now one of the least of the ills against which the slum child needs protection the value of a clinic lies not in doling out free cod liver oil but in guarding the health of the healthy infant by advice and those regular and

frequent examinations which alone can forestall illness. So in England one massive dose of vitamin D will not excuse regular attendance at a clinic nor even save the trouble of giving the infant cod liver oil since this is still needed for its vitamin A and its very valuable fat.

There is only meagre work on the safety of massive single doses of vitamin D. Thus Palmén [195] gave 500 000 I U to seven non viable infants with congenital defects; at autopsy no changes were found in the kidneys, adrenals, liver, spleen, aorta or heart muscle, but too little time may have elapsed between treatment and death for calcification to have occurred. Houet [196] gave 600 000 I U to five normal infants; during the short time they were under observation calcium retention was not affected but there was some increased excretion of phosphorus. In rachitic infants 600 000 I U given by injection caused the blood calcium to rise to normal in twenty one days as against four days when given by mouth [197], presumably because of the slow absorption from the site of injection [102]. This suggests that it is safer to give large doses by injection—but only as long as the vehicle is a slowly absorbed oil [128]. But in spite of these rather slight pieces of evidence all being in favour of the safety of single massive doses, we are still unhappy about their use. Apart from the *prima facie* case against giving large amounts of a toxic substance, there is the observation by Turk [175] that renal infections are more prone to occur just after large doses, while in puppies [198] single doses of 450 000 I U cause renal and pulmonary calcification and also abnormalities in the unerupted teeth, which is reminiscent of the delayed dentition and stunted growth seen in infants on daily doses of only 1 000 I U (p 532).

Germany was the first country to use single massive doses of vitamin D both for the prevention and the cure of rickets, Harnapp [199] coining the phrase 'Vitamin D Stoss' therapy for this. From a study of one hundred infants he decided that protection against rickets for at least four months was given by 300 000 to 600 000 I U, though thirteen of sixty infants given 600 000 I U by Brockman [174] developed craniotabes within four months. Turk [175] prevented rickets in thirty premature infants for six months by oral or intramuscular doses of 200 000 to 400 000 I U, while most of his untreated controls did develop rickets. In Scandinavia Palmén [195] treated two hundred premature infants with 500 000 I U—none developed rickets and none appeared to be harmed. In America Rambar and others [200] successfully treated twelve infants with a single oral dose of 600 000 I U or a monthly dose of 100 000 I U for seven months; no controls were studied and their results were only confirmed by X rays. Wolf's first observations [201] on the effect of large oral doses were partly controlled, since of his sixty two infants eighteen had rickets at the beginning of treatment and none had rickets nine months later, after having had two doses of 600 000 I U with a three to six months interval between. In a second paper Wolf [201] advises 50 000 I U at one and two months of age and a third dose of 600 000 I U at three months of age. Of twenty one infants given these doses two developed rickets, radiologically confirmed just before the third dose—and this at an age when X rays are more prone to miss than to disclose rickets. As Wolf ignores craniotabes and mild beading of the ribs and did no phosphatase estimations, the incidence of rickets in his cases may have been considerably higher than he states (p 548). He also includes breast fed babies in his series, because it has been shown that vitamin D is not secreted by the breast.

Krestin [202] is the only English worker who has used single massive doses. He gave 300 000 I U by mouth to forty three infants under one year of age. Of these three developed rickets within six months. In a comparable group of fifty six infants whose mothers were supposed to be giving them 1 500 I U daily, five developed rickets. The single massive dose had no adverse effect on growth, gain in weight or liability to infection.

Children between the ages of two and twelve should have two teaspoonfuls of cod liver oil in the winter when sunlight is scarce. If the parents have already warned the children it is unpleasant or if the children genuinely dislike the taste the classical method of floating it on peppermint is excellent or of taking a pinch of salt on the tongue before the oil. Concentrated fish liver oil capsules may be taken as a last and expensive resort. Of course ultra violet irradiation can be given instead of cod liver oil though with more trouble and cost (p 536).

Boarding schools—both boys and girls—often give very bad diets especially from the point of view of the costly foods rich in vitamin D at ages when they are most needed. In examining children who live at a boarding school for two thirds of the year the probability of cod liver oil being needed must be remembered.

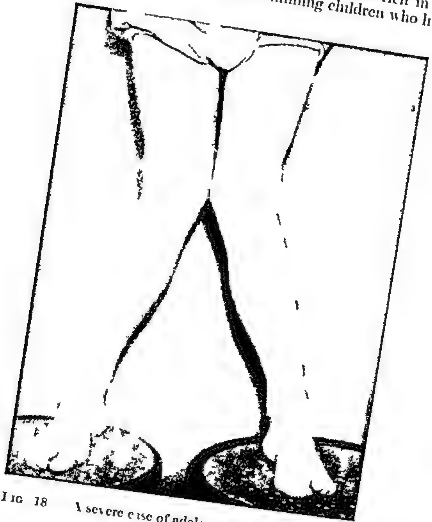


Fig 18 A severe case of adolescent rickets in England

Puberty and adolescence with its sudden growth impulse especially needs cod liver oil since mild rickets or osteoporosis (Fig 185) may occur at this period.

During pregnancy and lactation vitamin D is required both for the mother and for the child. The results of a deficiency are well seen in osteomalacia. McLennan [203] suggests that mild osteomalacia may explain why some women who have had no difficulty in earlier labours may appear in later ones to have a slight contraction of their pelvis. About 600 to 800 I U daily appear to be ample to allow the pregnant or nursing mother to maintain her own mineral reserves [205 206 207]. If the nausea of pregnancy is increased by concentrated fish liver oil capsules then calciferol may be necessary since in tablets it has no taste. Children born of mothers deficient

in vitamin D have been reported to have unduly soft skulls and poorly calcified bones [204] and to develop rickets or even to be born with rickets [208 209] On the other hand Finola and his colleagues [210] give the warning that too much vitamin D during pregnancy may cause excessive calcification of the fœtal bones of the head thus making birth difficult This has been emphasized to us by London gynæcologists who also complain that unduly large babies may be caused by the current craze for large doses of vitamin D

The adult needs are uncertain—most adults apparently get enough from their food and the sun to prevent obvious deficiencies But it seems probable that most diets lack some vitamin D breast milk often contains very little

FIG 186 Fœtal rickets in a Chinese baby photographed on the day of birth Note the rickety rosary and vertical grooves (*See also* Figs 182 and 189)

(p 543) while senile osteoporosis is fairly common and appears to be due to a mild vitamin D deficiency [211] Reed and his collaborators [111] have reported that huge doses—150 000 to 200 000 I U daily—given to men doing sedentary work increased their general well being weight and muscular power The men previously had been taking only about 100 I U daily and much The doses employed were in fact unphysiological but this does not detract from the implication that more vitamin D is desirable in the adult s diet Probably 300 to 600 I U a day should be taken

In old age the value of sunlight is often lost, since the old keep indoors, and the appetite may be small. According to Meulengracht [211] supplementing the diet of the elderly with cod-liver oil should prevent osteoporosis and the brittleness of the bones which so frequently leads to serious fractures, which, in the femur, for instance, may end fatally.

DISEASES DUE TO DEFICIENCY OF VITAMIN D

RICKETS

Lack of vitamin D is the cause of rickets.

The grotesque deformities of the rachitic dwarfs of a century ago are now so rare that one is apt to forget that mild rickets is still one of the commonest deficiency diseases. Probably between one-third and one-half of all the infants and children in England have or have had rickets. Reports on the incidence of rickets give such different figures because the different authors have based their diagnosis on different criteria. Rickets is now so mild a disease that its clinical recognition is often difficult, especially after infancy. This has led not only to an unjustified belief that clinical reports on the frequency of rickets are quite valueless, but also to undue reliance being placed on radiological reports. Actually the only completely reliable reports are those based on the level of the serum phosphatase and confirmed by clinical or radiological or post-mortem examinations. Such confusion, however, now exists about the incidence of rickets that some recent reports need to be discussed in full.

X-rays (p. 559) are not so valuable as is generally believed. They were the basis of the widely quoted survey on rickets, published in 1944, which was carried out in Great Britain during the first two months of 1943 on 4,818 urban and rural infants between the ages of three and eighteen months by the British Pædiatric Association for the Ministry of Health [212]. Each infant was examined clinically and also had one wrist X-rayed. Clinically thirty-three and one-third per cent. of all the infants had active or healed rickets, while in some towns the figure was above sixty per cent. These figures were, however, completely ignored when assessing the results of this investigation on the grounds that they were quite unreliable both because only five per cent. were confirmed by X-rays and because half of the 1·7 per cent. of infants who had positive X-rays were not diagnosed clinically as having rickets. But mild rickets in infants shows itself only in the skull and the costochondral junctions, the wrists not beginning to be affected until toward the end of the first year. Since only the wrists were X-rayed most of the younger infants, and many of the older inevitably appeared to be radiologically normal however clinically definite was their craniotabes or the beading of their ribs. In the final summary no attention was drawn to such considerations nor to the uselessness of the figures which were given. "The (calculated) rate of incidence of rickets diagnosed radiologically for children between three and eighteen months of age was two and a half per cent. before six months of age, four per cent. during the first year of life, and negligible over this age." The clinical finding that thirty-three and one-third per cent. of infants had active or healed rickets is, on the other hand, probably correct, agreeing with the other reports discussed below and most of the investigations made during the last ten years; these are summarized in the report which has just been discussed.

An analogous investigation to the above was carried out by Krestin [191], who during the autumn, winter and spring months of 1942 and 1943 examined clinically and radiologically one thousand healthy children between the ages of two months and five years who were in nursery schools in Preston. Of five hundred and eighteen infants below the age of two years eleven per cent. were diagnosed as having active rickets by X-rays and 36·5 per cent. by clinical examination. After the age of eighteen months rickets steadily declined till

between the ages of four and five years it was clinically present in only 16 4 per cent of seventy seven children The post mortem examinations discussed below suggests a higher incidence in this last age group and also confirm the

mild rickets

558) give the most reliable figures

for the frequency of

on many aspects of

is that of Corner [19

on infants in Bristol which lasted from September 1939 to May 1941 Eight hundred and twenty infants between the ages of two months and two years, being a representative sample of the healthy and sick population, were examined clinically and the plasma phosphatase was estimated in seven hundred and ninety seven X rays and post mortem examinations were also carried out in a few cases Rickets was not diagnosed unless the clinical findings were confirmed by an increase in the plasma phosphatase or by X rays or by autopsy Definite rickets was present in 31 4 per cent of all the infants and a further 12 2 per cent showed doubtful or very mild evidence of rickets At six to nine months of age 52 0 per cent of infants had rickets and at twelve to eighteen months 48 0 per cent Below six months the diagnosis was definite in only 26 2 per cent, but in another 17 3 per cent the serum phosphatase was either definitely raised or slightly raised and accompanied by doubtful clinical signs these figures may be unduly high since Corner included some infants whose phosphatase levels were probably within normal limits [215]

Clinical diagnosis (p 552) used alone for assessing the frequency of rickets in infants under two years of age has already been discussed, while reviewing surveys based on other forms of diagnosis The British Pædiatric Associa-

her confirmed finding of 31 4 to 43 6 per cent, does most strongly support the validity of investigations on infants based solely on clinical observations

Post mortem examinations for the estimation of the frequency of rickets have only, in recent years, been used by Follis, Jackson, Eliot and Park [217] who in 1943 published the results of consecutive post-mortem examinations on two hundred and sixty children between the ages of two and fourteen years who died from whatever cause in the Harriet Lane Home of the Johns Hopkins Hospital in America Histological studies, the validity of the technique being carefully confirmed, were made of the shafts and costochondral junctions of the middle ribs In these two hundred and sixty children active rickets was present in roughly forty per cent at all ages between four and fourteen years, and in sixty per cent during the third year of life Half the children of this whole series who died from an acute illness, lasting at most two weeks, had rachitic changes which were too extensive to have developed during their illness So it seems probable that rickets is as common in healthy as in sick children

It is of considerable interest that X rays were taken of all the ribs which were removed and only five were positive though one hundred and seven

the five positive X rays were

that dealt with in the above

nearly half the healthy adult

population of England must have had rickets and yet do not appear to have permanently suffered But mild rickets is of great importance Firstly it is a sign that our infant and child popu

the rachitic infant is one who is apt

is not going to grow to his full statur

patients whose chests and legs are mildly deformed, and whose pelves are

sufficiently contracted to make child birth difficult In fact, if the importance of the diagnosis and treatment of very mild rickets is not immediately obvious, yet taking the long view it is of the greatest value

Contributory Causes of Rickets In temperate climates the lack of sun and the expense of food rich in vitamin D often cause the milk of nursing mothers and the diet of children to be deficient in vitamin D (p 542) It is not, therefore, surprising that the time of year at which rickets is most common depends entirely on the amount of sunlight Thus rickets shows a sharp increase in late winter and early spring when the reserves of vitamin D are low both in nursing mothers and in food, and have not yet been replenished Rickets, however, may occur with ample sunlight especially in dark skinned people suffering from severe malnutrition [218, 219]

An inherited disposition to rickets is generally accepted, though, of course, accurately to disentangle all the factors which ameliorate or intensify the disease in man is impossible [208] Still [220] points out that sometimes the osseous sometimes the nervous or respiratory systems are most affected in early rickets, this predilection of the disease for a special system often being found in all the children of the same family It seems probable that the negro requires more vitamin D than natives of northern countries [217]

FIG 187 Early rickets in a Brahmin who is almost in the "Budha position"

Turning to the individual infant by far the most important personal factor in the causation of rickets is the rate of growth It is so important that the position has been summed up as "no growth, no rickets"

Breast feeding during the first six months of life reduces the incidence of rickets but increases it after this age compared to infants weaned on to a good diet A poor antenatal diet has an adverse effect which lasts at least until the end of the second year, this prolonged effect being probably due to the antenatal diet reflecting the diet which is given to the weaned infant It makes little difference whether artificially fed babies are given milk, or dried milk or sweetened condensed milk [190]

Premature infants and twins are very prone to develop rickets, partly because they grow fast, partly because they start life deficient in bone salts and vitamin D, with a digestion which cannot easily make up the deficiency

Infections, chronic and acute, are generally believed to make rickets worse However it is an old observation that children with tuberculous glands seldom develop rickets [208] and in Corner's study [190] of eight hundred and twenty infants rickets, which was present in 31 4 per cent of these, was commonest in the healthy and lowest in the severely ill In post mortem studies on children between two and fourteen years of age rickets was not found associated with any particular disease apart from lead poison

ing [217] Presumably the healthy infant is more prone to rickets than the sick because he grows faster

associated with rickets or rather causes raniotabes which for so long has been ilitic in origin—if not due to osteogenesis imperfecta or hydrocephalus—is discussed on p 553 There it will be seen that probably it is purely a rachitic condition though it may be an example of the predilection of an infection for certain tissues being determined by the especial susceptibility of such tissues to a deficiency of a particular vitamin such as is seen in the occurrence of bronchitis in infants suffering from lack of vitamin A

The age of onset of rickets varies slightly in different countries due most probably to the *maternal diet which starts the infant off either with an*

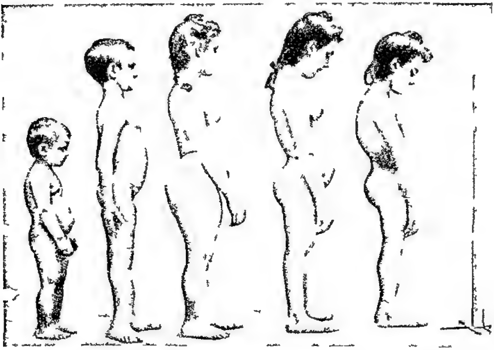


FIG 188 Five French children in one family with rickets aged 6½ 11½ 13½ 14½ and 17 Note bossing of the forehead bent tibiae curvature of the spine and protuberant abdomen (*See also* Fig 178)

adequate or poor supply of vitamin D and bone salts In the post war famine in Vienna where the mothers themselves were poorly nourished rickets in infants only three months old was a common finding while in

enerally thought to be fairly
gures which have been given
rickets (p 548) After six

months rickets increases until the middle of the second year and then declines as the first wave of the growth impulse is spent and the child tends to eat a wider diet and to spend more time outdoors

Rickets however can occur at any age Infants (Figs 186 and 189) have been born with rickets [208 209] and late rickets or juvenile osteomalacia may occasionally be found in older children (Fig 185) especially during periods of rapid growth such as at the second dentition and puberty a wartime example in England is described on p 537 At this age the picture hovers between rickets osteoporosis and osteomalacia depending on how much active growth is still going on, the minerals in the

diet and the severity of the deficiency (Fig 185) After growth has finished a deficiency of vitamin D causes osteomalacia which is only adult rickets modified by the absence of growth (p 564) Girls are slightly more susceptible to rickets than boys and dark children more than blond [208]

The General Symptoms of Rickets The clinical picture of rickets is too often painted as if the bone changes alone were important but rickets is a disease which affects the whole body so that even if the osseous system were ignored a very definite disease picture would still remain

The rachitic infant is a tired restless unhappy creature, by day he is fretful by night he sleeps badly throwing off his bedclothes as he twists and turns He impatiently rolls his sweating head on his pillow even to the

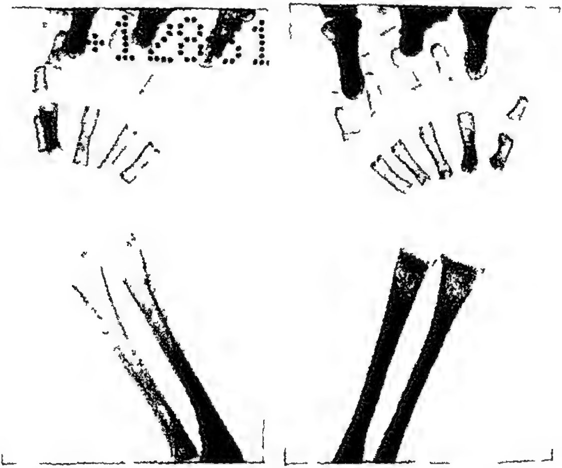


Fig 189 Fetal rickets in a Chinese baby X rayed on the day of birth Note the uneven frayed diaphyseal ends of the radius and ulna and the cupping of the latter (*See also Figs* 182 and 186)

extent of wearing away his hair His bowels are often constipated with sudden attacks of diarrhoea His stomach is distended partly because the intestines lack tone and are blown up by carbohydrate fermentation from his faulty diet partly because the liver and spleen are forced down by the deformed chest and partly because the abdominal muscles are flabby This flabbiness affects all the other muscles of the body while at the same time the ligaments of the joints are lax, so that the limbs may be twisted into bizarre positions The Buddha like position commonly seen in statues of the holy men of the East with the soles of the feet lying upwards is probably the result of rickets and osteomalacia A very mild tenderness of the bones is common causing the child to cry if roughly handled But any marked tenderness is more suggestive of scurvy.

Catarrhal infections are common they are not due to the rickets but

rather to the unhealthy life and poor diet of these children, a diet which, among other things, is probably grossly deficient in vitamin A

Spasmus nutans is a rare complication when it occurs it is generally with mild rather than severe rickets The child slowly nods or shakes his head when it is unsupported, and at the same time has a rapid, very slight or more obvious nystagmus, which is almost unique in often being unilateral When the two eyes are affected their movements may bear no relation to each other and move in any direction and manner Hippus—rhythmic contractions of the pupil—is also sometimes seen Spasmus nutans is generally found in the first year of life, starting in the winter, and disappearing in a few months Its cause is obscure It is not due to the infant living in the twilight of a cellar or dark room, such as would cause miner's nystagmus, because it is found in infants who live in well-lit rooms

Anæmia is common, being generally of the normocytic type; it seems to be rather the result of general ill health or bad food than an essential part of rickets though in animals vitamin D, especially from fish liver oils, slightly increases the absorption of iron and the formation of hæmoglobin [122] The liver and spleen may be just palpable This has no significance, being due to the deformed thorax pushing down the viscera A really enlarged spleen suggests syphilis Von Jaksch's anæmia, or pseudo-leukæmia infantum, is often associated with rickets for some still obscure reason

The usual infantile milestones are passed late The teeth are often not cut until after the first year (p 570), crawling and walking are delayed, or if walking has started the onset of rickets "takes the child off his legs"

Mental development is unaltered, though severe cases may show the precocity which is common in invalid children who of necessity are too much in the company of adults

The Bony Changes of Rickets. The bony changes which may occur in rickets are craniotabes, a large square shaped head, late closure of the fontanelle, beading of the ribs and deformity of the chest, bending of the long bones enlargements at the epiphyses, and fractures

To understand these changes it must be remembered that —

(1) Rachitic changes are greatest where growth is most rapid

(2) Deformities are the result of the combined action, on the bones, of gravity and the pull of muscles

Craniotabes is the earliest finding in rickets and is diagnostic of the disease when associated with beading of the ribs [190] While it is very common in rachitic infants under nine months, it is often completely absent in severe cases in older children This is partly because the growth of the skull is extremely rapid in the first months of life, and partly because when children start to sit up there is no longer continuous pressure from the pillows on the skull

In typical craniotabes round or oval unossified areas are found in the skull which yield like parchment under the pressure of one's fingers, giving a crackling feeling These areas vary in size from those which can be just felt with the tip of a finger to those which appear to fit the entire ball of the thumb They occur in the bones on which the weight of the head rests as the infant lies in his cot, the upper occipital, the posterior parietal, and sometimes in one upper temporal bone if the infant lies much on one side They do not join with the sutures, but often lie close to them

Confusion may arise either from the soft feeling of the skulls of some infants in whom calcification is delayed, or because craniotabes is generally stated to be a finding common not only in rickets, but also in congenital syphilis Probably craniotabes is always rachitic in origin, so that when it occurs in syphilitic infants they are suffering from two diseases Still [220] points out that the condition in syphilitics will clear up in seven or ten days with vitamin D and no anti syphilitic treatment, and also that craniotabes is frequently associated with laryngismus stridulus, which is a rachitic and

not a syphilitic condition. Also syphilis tends to make the bones harder, not softer. In osteogenesis imperfecta the whole of the vault of the skull and not only small areas in its posterior half is largely membranous, while in hydrocephalus the size of the head prevents confusion.

The persistence of the anterior fontanelle is common and should suggest rickets if it still measures an inch each way at the end of the first year or persists after the age of two.

The enlargement of the skull is difficult to explain. The four crosses over the frontal and parietal eminences which give the square hot cross bun effect are due to the heaping up of osteoid tissue in these regions.

The chest also suffers in early infancy when the ribs are growing rapidly and are very soft. The pull of inspiration drags in the sides of the chest so that the sternum is left sticking forward like the keel of a boat. At the same time the lower ribs flare out, being supported from collapse by the liver and heart, leaving Harrison's sulcus above them. These deformities of the chest are serious. Full expansion of the lungs is impossible so that even mild catarrhal infections are liable to end fatally because the flaccid cage of the ribs collapses more and more with any obstruction to inspiration.

Fig. 100. Rickety Rosary.

Beading of the ribs, the rickety rosary, is a nearly constant finding after the age of six months or even earlier, but not one on which much reliance as a diagnostic sign can be placed in mild cases. Corner [190] in her extensive study of eight hundred and twenty infants found that one third of all infants with mild beading and one eighth with definite beading had not got rickets, judged by the level of the plasma phosphatase; beading associated with craniotabes was diagnostic. The beading is the expanding osteoid tissue at the junction of the rib and its cartilage, being most obvious over the fifth, sixth and seventh ribs. Some slight swelling at the costo-chondral junctions in young infants is normal and has no rachitic origin. The ribs in severe cases may be displaced backwards so that no beading is felt. Posterior beading is caused by greenstick fractures at the angles of the ribs.

The arms become deformed when the child starts to crawl or sit upright. In the little . . . himself and as the weak muscles of his back . . . curls forward supporting himself on his arm . . . der the strain, while the already enlarged radial and ulna epiphyses broaden still further. The clavicle is bent upward and forward at its inner third by the pull of the neck muscles at one end and the drag of the arms at the other. The fingers sometimes show sausage-like enlargements due to the shafts of the phalanges swelling from osteoid tissue, while the joints remain a normal size.

The femur develops a forward curve from the weight of the legs when the child is sitting in his mother's lap, while later on, if he manages to walk, the

lower third of the tibia tends to bend outward and forward and the femur to bend outward But at this stage any deformity of the legs may occur, increased by the lax ligaments of all the joints

Pelvic distortion, is, however, the most serious result of rickets It may make child bearing in later life impossible except by Cæsarean section, so that in girls every effort must be made to insist on thorough treatment, and above all complete rest from walking until the pelvis is firm enough not to bend under the weight of the body

Greenstick fractures are common and may be overlooked, being often

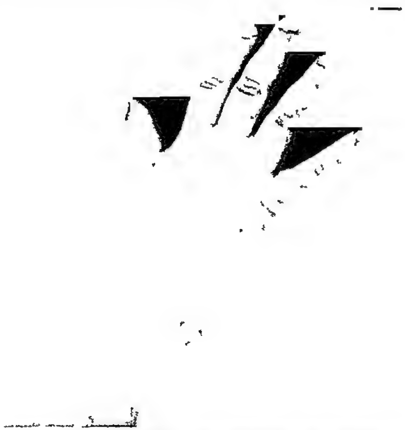


FIG 101 Rickets in an English infant Note the deformity and enlargement of the epiphyses at the wrist (*See also* Fig 102)

painless and giving no more deformity than may have already arisen from the twisted bones

Dental caries is not caused by rickets (p 572)

Atrophic rickets is the name given to rickets where osteoporosis is pro

Spasmophilia The nervous symptoms of rickets are so important that they are often discussed as if they were a different condition, and it is true that some of the worst cases, those with generalized convulsions or prolonged attacks of tetany, show only very slight signs of the underlying rachitic condition Indeed, it may be the convulsions which first cause a suspicion that rickets is present

The proportion of rachitic children who suffer from spasmophilia varies greatly in different places The underlying mechanism is a low blood calcium,

where the calcium of the diet is high the tendency to spasmophilia should therefore be slight, but as yet this has not been investigated Boys are affected more often than girls and the condition may occur at any age, even in infants a few weeks old

The irritability of the nervous system may show itself in many ways The most trivial manifestations are sudden starts with a cry as if the child were suddenly pricked, or for a moment he turns his eyes up, or he cries out in his sleep, rolls over and is quiet again Facial stiffness is also quite common The muscles of the child's face are in spasm, so that when he

FIG 192 Rickets in an English infant Note the bowing of the tibiæ, and the enlargement and deformity of the epiphyses at the ankles (*See also* Fig 191)

cries the face gives an impression of being stiff Change of expression is limited, lacking the finer shades

Or, again true tetany may occur, the hands and feet being drawn into the typical position, with the thumbs pressed against the fingers, which are held rigidly straight though flexed at the carpo metacarpal joints with the wrist slightly bent The feet are extended while the toes are bent down and bunched together These spasms may last for seconds or hours They appear to be only painful when they start, so that children will often go on clumsily playing with their toys during an attack If the spasm lasts for some hours both the hands and feet may swell from static congestion

Laryngismus stridulus is, however, a more frightening symptom The child on waking, or when he starts to cry ... no very obvious cause suddenly gets a spa ... eath, appears terrified, and goes blue in the ... passes and he draws in

his breath with a long whoop Sometimes the child does not recover so simply but goes into general convulsions

General convulsions may occur for no reason but usually the child has had an attack of diarrhœa or a mild feverish upset The attack starts with the child going into tonic stiffness which passes into clonic convulsions After a minute or two the attack passes the child being again normal but exhausted Generally these attacks occur at long intervals but they may follow each other so rapidly that the child does not regain consciousness between each one

There is a definite danger to life in infantile convulsions so that it is important to recognize and treat the early mild symptoms of spasmophilia before generalized convulsions occur The treatment is discussed on p 562

Examination of the child may disclose any of the classical signs of latent tetany due to an unusual excitability of the motor nerves—Chvostek s sign or twitching of the muscles of the face when the facial nerve in front of the ear is lightly percussed Trousseau s sign or compressing the arm or leg by encircling it with the hand and squeezing when the hand or foot goes into the position assumed in attacks of tetany Erb s sign or undue excitability

FIG 193 Tetany in an English infant with rickets Note the position of the hands and their swelling from static congestion

of the motor nerves to electrical stimulation—but the necessity for electrical apparatus and experience in its use vitiates the value of this test

Other causes besides rickets may cause infantile convulsions Acute fevers and infections of the brain and spinal cord often start with convulsions In these conditions the child between its convulsions is still ill while in spasmophilia it is well Trivial causes like constipation or indigestion may cause doubt for a moment and middle ear disease must be remembered Any infection may flare up a latent tendency to true spasmophilia

Idiopathic epilepsy is rare at this age and will not yield to the treatment for spasmophilia

Lead encephalopathy may closely simulate spasmophilia the earlier symptoms being vomiting and convulsions Mild cases often occur in the spring when the increased supply of vitamin D from sunlight enables children to absorb more lead from the paint of their toys etc since lead absorption is increased by vitamin D [222 223] Lead poisoning is said to cause rickets [217] and so presumably would poisoning by any of the metals mentioned on p 527 which form insoluble phosphates

Aids to Clinical Diagnosis of Rickets In severe cases of rickets the diag

nosis is simple but in early cases doubt may be felt over the significance of mild beading of the ribs dubiously enlarged [illegible] closure of the fontanelle mild spasmophilia [illegible] bone changes recurrent attacks of bronchiti[illegible] to walk and general poverty of well being and [illegible] it is wise to remember that mild rickets may be the cause of many rather vague symptoms Rickets is a disease of general metabolism

Whenever there is hesitation over the clinical diagnosis of active rickets the alkaline serum phosphatase should be estimated this definitely confirming or disproving the diagnosis since other generalized bone diseases and hepatitis which may increase the serum phosphatase are of academic rather than

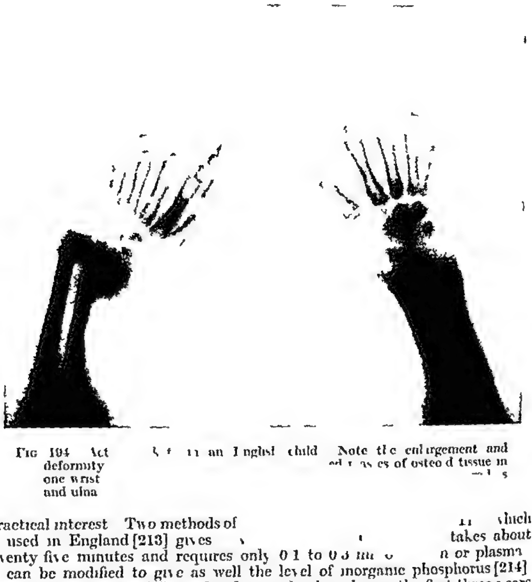


FIG 194 Act[illegible] an English child Note the enlargement and [illegible] deformity [illegible] one wrist [illegible] es of osteo d tissue in [illegible] and ulna

practical interest Two methods of [illegible] which is used in England [213] gives [illegible] takes about twenty five minutes and requires only 0 1 to 0 [illegible] or plasma it can be modified to give as well the level of inorganic phosphorus [214] According to Gray and Carter [215] normal values during the first three years of life are 11 to 20 units, 17 units being the average and 20 units the upper limit of normal Between three and five years the average is 12 units with a range of 8 to 18 units In adults values vary from 0 to 10 units Corner [190] however put the average at all ages as being 3 to 10 units 15 units and over being diagnostic of rickets But from the results of investigations on apparently normal children from the changes with age in Bodansky units and especially from the results of giving vitamin D to children with levels of about 20 units it seems definite that the figures of Gray and Carter are the more correct In early rickets levels of 30 to 40 units are usual but the same

levels may be found in more severe cases, so that high phosphatase levels are diagnostic of rickets but not of its severity. Levels in severe rickets under treatment fall, then rise and then fall again, while in less severe rickets there is a steady fall from the beginning [215], but normal values may not be reached for three months or longer. In the U S A. "Bodansky Units" are generally employed, measured by a technique which requires 2 ml. of serum and takes over an hour to complete [216]. It is said that 20 Bodansky units is the average for premature infants [178], while for normal infants the average is 7 1 units on the third day of life, rising to 13 units at the third or fourth month with a fall to 11 5 units at the age of three years and a further fall to 1 4 to 4 units in adult life [215]

The blood calcium in infants is normally 10 to 11 mg per 100 ml, and in the usual type of rickets is little if at all below this level. In the "low calcium" type of rickets associated with spasmophilia this calcium may be as low as 4 mg but is more often between 5 and 7 mg

The inorganic phosphorus is normally 4 5 to 5 5 mg per 100 ml, which commonly falls in rickets to 2 to 4 mg, 4 mg being generally taken as the dividing line between the normal and the rachitic. But a low level of either phosphorus or calcium is by itself no proof of rickets, when, however, the product of the two is below 40 this is definitely suggestive of active rickets

FIG 195 Healed rickets in the child shown in Fig 194 The X ray, taken five years later, is of the wrist which was most affected

The typical X-ray appearances of the bones in active and recently healed rickets have been described on p 530 For the ...
however, X rays have several drawbacks
in infants under one year of age are found
rounding tissues prevent these being satisfactorily X-rayed As a consequence the wrist has to
the end of the first year
that rickets is not preser
end of the ulna shows "

end of ' ' The increased width of the cartilag hough spreading up along its sides tl richondrium may be visible The early changes in rickets must be diagnosed by experts and even these are liable to differ in their opinions Much confusion is caused by the ends of rapidly growing bones showing slight irregularities which suggest rickets though they are really normal, being due to calcification being so rapid that it is not quite uniform [224, 225]

Differential Diagnosis of Rickets In congenital syphilis the X ray changes are moth eaten epiphyses and periostitis while clinical stigmata are probably present Of course rickets being common in congenital syphilis may complicate the radiological and clinical picture

In osteogenesis imperfecta confusion will be avoided if the difference in the changes in the skull is remembered (p 554) and the blue sclerotics

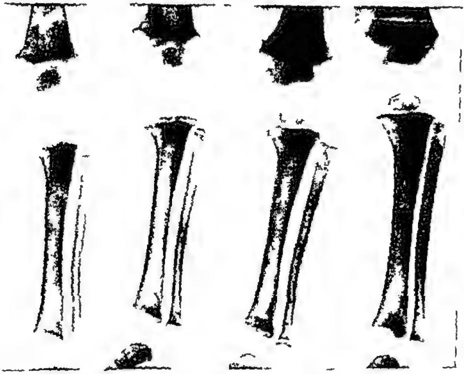


Fig 106 Rickets cured by cod liver oil

There is a frequent family history The X ray appearances are not those of rickets and the blood calcium phosphorus and phosphatase are normal

Chondro osteo dystrophy in older children will not be mistaken for rickets if an X ray is taken, since the epiphyses are fragmented and the lumbar vertebræ are deformed

Achondroplasia leads to an unduly long body compared to the short legs and arms the hands are like star fish the head is domed X rays and the usual biochemical investigations confirm the diagnosis

Scurvy causes acute pain when the child is gently moved and there is bruising bleeding gums if the teeth are erupted, red blood cells in the urine and subperiosteal hæmorrhages and epiphyseal separations—the latter never occur in rickets, while pain is never severe and there is no hæmorrhagic tendency Rickets and scurvy can of course occur together

Renal rickets and other forms of ' rickets are discussed on p 562

Treatment of Rickets The amount of vitamin D required for the treatment of most infants with moderately severe rickets is about 1,200 I U a day Individual needs vary greatly The correct dose is that which cures

the rickets some children with "resistant rickets" a strange condition discussed on p 562, requiring enormous doses

The various sources of vitamin D have been discussed on p 538 so that here it is only necessary to point out that up to 1,200 I U daily can be given as cod liver oil, but that higher doses need the use of one of the concentrated fish liver oils

Some physicians advise very large amounts of vitamin D by that means hurrying the cure There seems no advantage in most cases over a lower dosage and slower recovery The only exception is in infants where the weakness and collapse of the ribs is threatening death from pulmonary infections [226] Here doses of 50,000 units a day may be given in order to calcify the ribs with the greatest rapidity, X ray and blood examinations must be used to check the results If the infant is too marasmic to take a normal amount of milk, calcium phosphate gr 10 should be given daily to supplement the mineral intake

The use of artificial sunlight as an alternative or aid to cod liver oil has been discussed on p 536

Mineral salts given without vitamin D are valueless and calcium preparations containing neither magnesium [227] nor phosphorus [79, 80, 81] are definitely injurious

Improvement is hard to judge clinically The changes in the bones fade away too slowly to be of value in gauging the success of treatment except where craniotabes is present, when healing should be obvious in two or three weeks The general condition of the child furnishes some evidence He is better in

X ray

treatmen

faint line of preliminary calcification should appear running across the clear epiphyseal cartilage just beyond the end of the diaphysis The further X ray changes due to healing have been described on p 530

Phosphorus and calcium estimations are of value, within two weeks they should be approaching normal

The blood phosphatase levels approach the normal more slowly than those of the minerals, falling in two to three weeks but not becoming normal for about three months or even longer [215] In mild cases the fall toward normal is continuous from the beginning but in severe cases this fall may be interrupted by a brief rise [215] A fall in the phosphatase is the best guide that healing has started [30] and in doubtful early cases is proof that the diagnosis of rickets was correct [215]

Cœliac Rickets Cœliac rickets is only rickets complicating cœliac disease a common complication because the inability to absorb fats reduces the amounts of both vitamin D and calcium acquired by the body Treatment consists in giving calcium lactate in large doses by mouth and also vitamin D Irradiation is ideal (p 536) but if it is too costly a highly concentrated fish liver oil containing little fat or even tablets of calciferol or of synthetic vitamin D_3 must be used

Scurvy—Rickets This is a combination of scurvy and rickets the usual treatments for each condition being required

Treatment of Rachitic Deformities Rest is the essential treatment both for

since only at rest is the weight

and insistence on regular rest after lunch is sufficient Where deformities have already occurred splinting may be necessary to prevent the child crawling or walking until the bones have become recalcified This is absolutely essential in girl babies, as their pelves must be protected from distortion General massage is invaluable when no exercise is taken, a masseuse can rapidly teach the mother how to give it

The body's power of remoulding deformed bone is remarkable, but where the deformity is gross, surgical correction may be necessary. The suggestion that the bones should be deliberately decalcified to such an extent that they may be bent back to a normal position is bad.

The prognosis in deformities is excellent, except in the pelvis which in girls may cause serious trouble over childbirth in later life. Some abnormality of the chest is liable to persist, and deformities acquired at puberty when growth is nearly over will not disappear of themselves.

Treatment of Spasmophilia. The immediate domestic treatment of convulsions is to put the child in a hot bath at a temperature of 100–105° F.

Calcium deficiency being the immediate cause of spasmophilia the level in the blood should be raised rapidly. Calcium chloride gr. 15 thrice daily in milk may be tolerated and is ideal, or calcium lactate gr. 30 may be given instead. In continuous convulsions 5 ml. of a ten per cent. solution of calcium lævulate or gluconate should be given intravenously if possible, but if not into the buttock. Calcium chloride, 1 ml. of a two per cent. solution, is excellent, but it causes ulceration unless it is given into a vein, and for this reason can only be used in an emergency when no other calcium preparation is available.

Sedatives should be given to all children who show any signs of spasmophilia. Vitamin D acts slowly and until it acts there is always the risk of general convulsions, which may even end fatally.

Rickets Due to Deficient Renal Tubular Reabsorption of Phosphorus. In this group of rare diseases the essential abnormality [228, 229, 234, 235, 243] is a failure of the renal tubules to reabsorb phosphorus. This causes a hypophosphatæmia which in its turn causes a chronic insufficiency of phosphorus for the calcification of bone. Since in ordinary rickets there is a similar hyperphosphaturia, etc., which is corrected by normal doses of vitamin D, and since in the rare forms of rickets correction may also be largely or wholely achieved by huge doses of vitamin D, it would seem that in essence both types of rickets are the same, only differing in the amounts of vitamin D required by that enzyme system of the kidney [235] which is concerned with phosphorus reabsorption.

The great general interest of this group of diseases lies in their being the only definite known examples of deficiency diseases which (*a*) are often inherited and (*b*) are not cured when the deficient nutrient is supplied in ample amounts, judging not only by its intake but also by its level in the blood. These two facts, bearing in mind that these diseases are curable by enormous doses of vitamin D, mean that a disease cannot be dismissed from the ranks of deficiency diseases and diseases which are curable merely because it is familial or, which is more important, because there is no response to treatment which judged by customary criteria should be successful.

Raised Resistance to Vitamin D (R. R. D.) or Resistant Rickets or Refractory Rickets. This condition is in its clinical picture ordinary rickets which fails to respond to vitamin D until the dose is a hundred or more times as large as that usually required. McCance [228], who coined the name Raised Resistance to Vitamin D or R. R. D., has written the best of several excellent reviews and papers on this subject [228–235].

The familial tendency in R. R. D. is very strong, siblings of both the patients and their parents frequently being found to be affected, though isolated cases occur. An onset in infancy is usual, but it may be delayed until early adolescence [228]—an age, oddly enough, at which spontaneous improvement often occurs [233].

The cause, as stated above, is a failure of the renal tubules to reabsorb phosphorus. There is no inability to absorb vitamin D from the gut, since blood levels may be over a hundred times the normal before there is healing of the rickets [230].

The diagnosis of R. R. D. depends on recognizing that what would be

ordinary rickets were it responsive to normal doses of vitamin D is in truth rickets but rickets responsive only to abnormal doses of vitamin D All the biochemical investigations which are of help in the diagnosis of ordinary rickets (p 537) are just as helpful in the diagnosis of R R D and unmask the same abnormalities The X ray changes [236] are also those of ordinary rickets but in spite of all this easily gleaned evidence which in sum can only mean rickets a diagnosis is often made of chondrodystrophy or osteogenesis imperfecta or hyperparathyroidism or even of muscular dystrophy, though these have few or no biochemical similarities with rickets

Treatment consists in giving as much vitamin D—which may be even a million or more I U daily [232]—as is necessary to control the rickets though this in sharp contrast to ordinary rickets may fail to correct entirely the hypophosphatæmia [229] further and again in contrast to normal rickets vitamin D must be given daily no effect being produced by very large doses at long intervals The dose may vary considerably from time to time and may fall abruptly at puberty A serious danger [230 231 233 236] which means that the child must be under constant supervision is that there may be little margin between the curative and the toxic dose Mackay and May [233] for instance describing two children in whom there was virtually no margin one of them even developing the osseous changes of vitamin D poisoning (p 583) Some pædiatricians urge that the urine should be tested weekly with Sulkowitch s reagent because an increase in urinary calcium occurs not only in those cases with hypercalcæmia but also in cases with normal calcæmia

But probably the

guide as to whet

further danger is sudden hypercalcæmia with serious renal damage when such children are put to bed after osteotomies or for any other reason since added to the tendency of all young patients to develop hypercalcæmia when they are immobilized is the hypercalcæmic effect of vitamin D The danger unfortunately is only slightly abated by stopping treatment a short time before any operation because the effect of vitamin D and a raised blood calcium may persist for weeks

The Fanconi and Other Syndromes [237 244] These conditions are in essence the same as R R D but the urine also contains glucose and often amino acids other abnormalities in its secretion may occur From the point of view of rickets the only germane excretion is the excessive phosphorus but inevitably the rickets of patients with these abnormal kidneys have been labelled with particular names though R R D with renal glycosuria etc would have been clear and logical Of these names the de Toni Fanconi Syndrome and Cystine Rickets are the best known the former implying rickets hyperphosphaturia renal glycosuria and often the excretion of amino acids—probably cystine—in the urine while the latter is the same To this group may be added the further abnormality of failure to acidify the urine and perhaps failure to form ammonia The same failure over acidification and formation of ammonia and with hyperphosphaturia but with no other renal abnormality is found in the Butler Albright Syndrome (renal acidosis or nephrocalcinosis) All these conditions save the last are often familial They would as regards the rachitic abnormality probably respond to vitamin D in sufficiently large doses [228] in spite of the belief of earlier workers who used too small doses that they would not indeed Stevenson [243] has reported one case with sugar and amino acids in the urine which had not only the rickets but also apparently the urinary abnormalities controlled by 100 000 I U daily

Renal Rickets This condition also known as renal infantilism renal dwarfism renal osteitis fibrosa cystica and renal osteodystrophy is the exact opposite of R R D being due to damaged renal function preventing the excretion of phosphorus with the result that it rises to such high levels

in the blood that calcium ionization is depressed and little calcification can occur Renal rickets is commonest in *mid childhood* It is characterized by rickets dwarfism albumin in the urine a *high blood phosphorus* and impaired renal function Vitamin D makes the *condition worse* by further mobilizing phosphorus from the tissues A high calcium low phosphorus

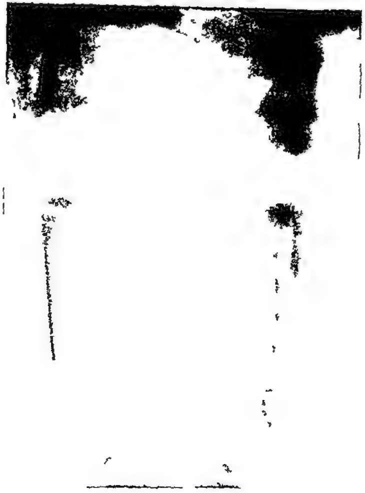


Fig 197 Renal rickets in an English infant Note the displacement of the distal tibial epiphyses and the slight calcification of the expanded osteoid tissue

diet may delay the hopeless march of the disease by aiding the bowel in the excretion of the excessive phosphorus

OSTEOMALACIA (MOLLITIES OSSIUM)

Osteomalacia of pregnancy hunger osteomalacia and probably many cases of senile osteoporosis are fundamentally all the same being due to a deficiency of vitamin D In the past they were not recognized as identical because the obvious cause appeared to be repeated pregnancies famine or old age rather than a common vitamin lack They are indeed the adult equivalent of low calcium rickets modified by the absence of growth

Osteomalacia is such a dramatic disease that sufferers have taken their place in myths and legends almost as if they were giants The French of the last century besides making the best clinical study of the condition [4] have collected many historical accounts [208 245, 246] The best known was a dwarf who in the sixth century lived to be three hundred years old but appears to have been more like a jelly fish than a man so soft were his bones

He could not move and was plagued by the teasing of dogs and children his compensation was his fame since he could be twisted into any position at will Then there was a woman called Supiot who attracted much attention in 1700 and another nameless who Ayant été d une taille tres elevees devint avant de mourir plus petit qu un nain Trousseau s account [4] of Madame Rehbin however is still the only perfect description of the disease and the perfect example of possibly the greatest physician s mastery of prose

Osteomalacia of Pregnancy This is now a rare disease in Western civilization though cases are still occasionally seen [203 247 248 249] and MacLennan [203] suggests that mild osteomalacia in England may be the reason why some women who have had no difficulty in earlier labours may appear in later ones to have a slight contraction of their pelves When

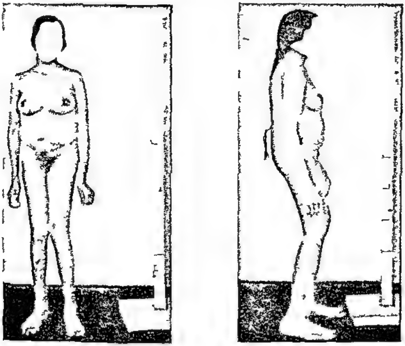


Fig [illegible]

osteomalacia was more common it was generally among young women who had had frequent pregnancies on bad food and no sunlight But it was also found during first pregnancies and then the mother was often over thirty

With each child the condition takes a step forward as the body is further drained of its minerals and vitamins And with each child the condition becomes more piteous the mother is more imprisoned in the house by pain and deformities her chances of earning more money of gaining more food more sunlight are curtailed her pelvis collapses further and further making her next confinement even worse than the last Sometimes her downward progress halts between her pregnancies sometimes she may even improve if fortunately she does not suckle her infant in the vain hope of thus warding off her tragic fertility

The gradual extinction over five hundred years of the Norse colony founded by Eric the Red in Greenland at the end of the tenth century was apparently due to the pelvic deformities of osteomalacia hindering childbirth

It is thought that the Norse colonists did not eat the local diet of fish and fish oils which is necessary to supplement the scant sunlight in Arctic regions [209]

Hunger osteomalacia This is found quite apart from pregnancy during periods of chronic famine Both sexes suffer and the elderly more frequently than the young who are more exposed to the sun The condition is endemic in areas where the diet is poor or custom keeps women indoors as in some parts of China and India There is a seasonal variation fewer cases being seen in the sunny part of the year when sunlight helps to eke out the deficient diet Rarely hunger osteomalacia is found in large cities where solitary old people live in proud self respecting poverty rather than apply for charity We have seen such an old woman who could only hobble along with difficulty and had lived for years alone in London

Steatorrhœic osteomalacia This is probably the commonest type seen in

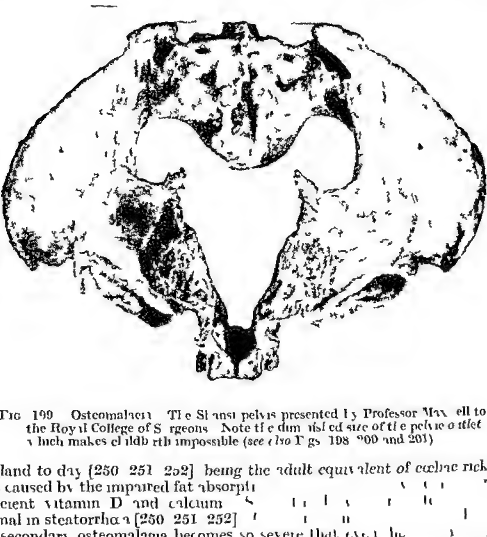


FIG 199 Osteomalacia The Sinansi pelvis presented by Professor Maxwell to the Royal College of Surgeons Note the diminished size of the pelvic outlet which makes childbirth impossible (*see also* Figs 198 200 and 201)

England to day [250 251 252] being the adult equivalent of cœliac rickets It is caused by the impaired fat absorpti[illegible] sufficient vitamin D and calcium [illegible] normal in steatorrhœa [250 251 252] [illegible] the secondary osteomalacia becomes so severe that eve[illegible] is forced to seek some definite reason for his patient s increasing pain and general misery Or the primary diagnosis may be made because of a megalocytic anæmia which fails to respond fully to liver therapy or to vitamin B_{12} [252]

Senile Osteoporosis This appears to be often a mild form of hunger osteomalacia and not as was formerly held a senile atrophy of bone [211]

Symptoms Diagnosis and Treatment of Osteomalacia The earliest symptom in osteomalacia is a vague tenderness or aching pain in the bones of the lumbo sacral region and hips which is worse when the patient walks It is generally called *rheumatism* As the condition progresses the tender

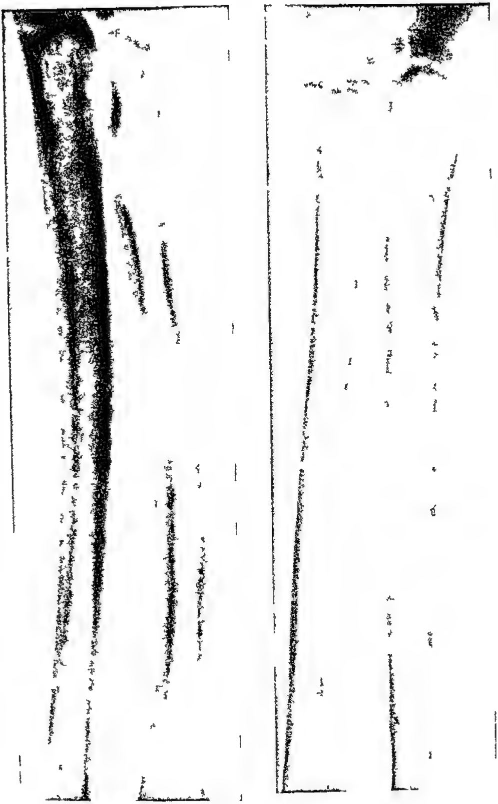


FIG. 900 English Osteomalacia FIG. 901

X-ray of bones of forearm (right) showing Looser's zone (left) control normal forearm

ness and aching of the bones spreads so that percussing the chest may cause the woman to cry out She may even refuse to be touched at all and wince when approached.

Bony deformities often occur with surprising rapidity. The woman appears to shrink She loses height partly by the bowing of her legs, chiefly by the softening of her spine, which may become twisted and always bows forward, so that the ribs pile up over one another like the spokes of a fan, even coming to rest on her pelvis The pelvis itself becomes grossly distorted until childbirth, from being difficult, becomes impossible Trousseau [4] found a woman for whom a sound had to be used instead of digital examination, the pelvic outlet was so small The legs twist and bend in any direction Generally in the old it is the spine, in the young the pelvis and legs which are most affected (Figs 198 to 201)

Fractures from trivial falls, from the normal pull of the muscles, or from no apparent cause, are common They heal slowly or not at all, false joints forming instead

Muscular weakness and flaccidity analogous to that of rickets occurs, on getting up the patient climbs up herself like a child with muscular dystrophy The adductor muscles of the thighs tend to go into spasm due to the aching of the bones (Fig. 198) The walk becomes a wide based waddle compounded of weakness, pain, and deformity.

Cataract, according to Maxwell and Pi [247], is found in at least fifteen per cent of marked cases The condition develops slowly, and in its early stages can only be diagnosed with a slit lamp. It is important to consider whether cataract in young and middle-aged women may not be caused by undiagnosed osteomalacia which is still only causing vague pain in the back and limbs Treatment of the underlying deficiency of vitamin D may improve the cataract

The teeth are not affected Taylor and Day [253] studied twenty-two Indian women with severe osteomalacia and found only thirty-four cavities in five hundred and sixty-five teeth Eight of the women had no dental decay whatsoever These observations are of great importance as confirmation that dental caries is not due to a deficiency of vitamin D (p 570)

Menstruation and fertility in women are not, unfortunately, affected The ovaries have been said to contain an abnormal number of follicles

X rays may show in the early stages little beyond a doubtful rarefaction of the bones of the pelvis or spine, but in the late stages the picture is unmistakable with the extreme osteoporosis of all the bones mimmg the skel[illegible] frosted glass, with the kyphotic spine and its [illegible] ollapsed vertebrae, with the trefoil pelvis (Fig [illegible] fractures and deformities *Looser's Zones* [254] or translucent ribbon like areas of symmetrical decalcification may appear before there is any definite osteoporosis in any condition where there is general decalcification such as occurs in R R D or resistant rickets [228], in osteomalacia and in cadmium poisoning [69] Indeed the first indication of a serious disease is often the discovery of these zones They occur most commonly in the axillary borders of the scapulæ and also in the necks of the femora, the pubic and ischial rami, the ribs and the proximal third of the ulnæ (Fig 200) Their cause is obscure, they may be the result of impoverished bone reacting to mechanical stress or to the proximity of arteries *Milkman's syndrome* [255] or "Multiple spontaneous idiopathic symmetrical pseudofractures" is only another and later eponym, better forgotten, for the condition first described by Looser

In all forms of osteomalacia the blood calcium is generally low, often to tetanic levels but it may be normal if the parathyroids, which are often enlarged, succeed in mobilizing enough calcium from the skeleton, when this occurs the blood phosphorus may be low The phosphatase is slightly [244] or markedly [251, 252] increased The startlingly light bones, which

are often so soft they may be cut with a knife or bent like cardboard largely consist of osteoid tissue, their enlarged cavity is filled with hæmorrhagic osteoid tissue or fat, their ash contains little calcium, though the phosphorus is, by comparison, high Magnesium is increased The urine on standing may form a phosphatic scum

The diagnosis rests on the dietetic history, the tenderness, weakness and deformities, X ray examinations, the levels of the serum calcium, phosphorus, and phosphatase, and the response to treatment Sometimes the pain in the ribs is so acute that this, combined with the bronchitis which is so common, suggests pleurisy, a suggestion which is only discarded when the pain is found to be acute all over the ribs, which not only are exquisitely tender but in their weakness crepitate beneath the light pressure of the hand

Death, unless treatment is given, is from pulmonary infections and the toxæmia from bed sores

The outlook as regards life is excellent with treatment, some recovery of stature and amelioration of the deformities may be hoped for

Treatment is with large doses of vitamin D Probably about 3,000 I U daily will be enough, but much larger doses may be required To supply the necessary minerals for the recalcification of the bone calcium phosphate gr 30 thrice daily should be given as well as plenty of milk, cheese and fish Radiography and calcium estimations should, if possible, be used to make certain adequate vitamin D and calcium and phosphorus are being given

Extra calcium may have to be given if tetany persists (see p 562) or if treatment causes the blood calcium to sink further because it is diverted to the bones, thus bringing on tetany

In the past ovariectomy has been strongly advocated [245] It is quite unnecessary and unjustifiable

DENTAL DECAY

There is such a widespread belief that lack of vitamin D is the chief cause of dental decay that it seems important to consider the subject in some detail Dental decay, or dental caries, is due to the destruction of the enamel and dentine of the teeth by saprophytic organisms Their growth and the progress of decay may be assisted by any one of several different factors The more important are (*a*) the use of refined foods, especially white flour and white sugar (*b*) the use of heated milk instead of raw milk (*c*) the failure of the individual to produce "immune saliva," and (*d*) possibly, but improbably a diet deficient in vitamin D

Refined Foods. The bad effects of refined foods have been observed for many years The most classical observations were made on the island of Tristan de Cunha When the island was visited in 1932 caries was practically non existent, especially among people of under fifty No food was imported, the islanders living entirely on the unrefined produce of the island In the next five years, however, a large amount of refined flour and sugar was left by visiting ships so that the inhabitants suddenly had added to their diet foods which they had never had before save in minute quantities at long intervals The result from the point of view of dental decay was tragic Barnes [256] observed that in only five years caries from being almost unknown in children was common, while in adults between forty and fifty years old it had increased by fifty per cent

If further proof is required of the effect of refined foods it is furnished by the teeth of the Bantu [257] These people had exceptionally fine teeth, but when they started eating refined European foods caries became as common as in England In one generation perfect teeth, which have often been considered to be an inherited racial characteristic, were utterly lost That it was the fault of their new foods appears certain because there was no other change in their manner of life which appeared to have any possible

significance The increase in dental decay in the island of Lewis also tells the *same story* [258]

It is generally stated that white flour and sugar cause decay by *clinging round* the teeth while coarse fibre containing flours and sugarless foods being less sticky are easily removed by the natural self-cleansing action of the mouth—the "detergent diet" of Sim Wallace The sticky white flour and sugar are decomposed by bacteria, thus producing acids which erode the enamel This appears to be true, but is not the whole truth about the bad effects of refined foods

The work of Osborn and Noriskin [259] showed that unrefined sugars *and cereals have a protective action against the destruction of enamel* When they incubated human teeth with saliva and white flour, *or refined sugar*, the enamel was dissolved If, however, wholemeal *flour or unrefined sugar* were used the enamel was hardly affected, especially with the *unrefined sugar*

The protective substance present in unrefined food has not yet been isolated, but its action is not dependent on any change of acidity in the saliva It explains the puzzling observations that dental decay may be absent in the mouths of native children whose teeth are always coated in sticky sugar from eating raw sugar cane all day [257]

Heated Milk Raw milk, that is unpasteurized and *unboiled milk, has* a very marked protective action against dental decay This was found to be so by Sprawson [260], who *noticed a sudden startling fall in caries in a* children's institution The only change which had been made was giving each child daily a pint of raw milk. Further enquiries among dentists confirmed that children brought up on raw milk—either cow's or goat's—were free of caries Sprawson [261] also points out that in the island of Pitcairn caries was very common, while in Tristan de Cunha it was very rare The only difference between the diets was that in the latter island raw milk was drunk though probably less vitamin D was taken

In the first edition of this book the contentious subject of pasteurization was discussed *in its wider aspects* As, however, one of the authors (F B) believes pasteurization is unwise while the other (F P) believes in its value, it seems better *to omit this wider discussion, referring the reader to the* sections in each chapter for the narrower issue of how pasteurization alters the individual vitamins of milk

Immune Saliva. Fish [262] has pointed out that some human saliva prevents decay and some does not Dog's saliva always does so while monkey's saliva varies as in man When Fish placed carious human teeth in a dog's mouth in a few days they were sterile, the saprophytic organisms which cause decay had been destroyed The same occurred if a carious tooth was incubated with human saliva from a man with no active caries But [illegible] did not sterilize the tooth nor

[illegible]

If it is the product of a healthy *general* [illegible] a good diet gives protection against caries in children, without having to postulate the [illegible] in some cases by [illegible]

Traces of f[illegible] the teeth Such teeth are very resistant to decay, even when [illegible] is so slight as not to be æsthetically objectionable [265–268] *It is possible* that the fluorine, excreted into the saliva, acts by inhibiting the growth *of* saprophytic organisms in the mouth in other words a form of immune saliva is produced

Vitamin D. In rachitic children the teeth tend to erupt late and decay early The late eruption appears to be a direct effect of too little vitamin D, since Speidel and Stearns [95] report that there is an optimum intake of

vitamin D above or below which eruption is delayed The early decay is probably due to the refined carbohydrate diet such children generally eat The teeth of rachitic children, may, however, be perfect. Both Taylor and Day [218, 219] and Wilson [269] have reported many Indian children with severe active rickets who had no caries, even when born of osteomalacic mothers Their diet was very coarse, this coarseness appeared to be the reason for the absence of decay

In osteomalacia, as has been pointed out on p 568, the teeth do not decay unduly though here the lack of vitamin D and calcium is profound.

Vitamin D is therefore not *necessary* for good teeth in either the fœtus or the child or the adult. But the question arises as to whether vitamin D is *valuable* in helping to protect the teeth To give the answer before the evidence the addition of extra vitamin D to the diet appears in some cases to decrease dental decay, but we believe this to be due to the improvement in general health brought about by removing a mild deficiency of vitamin D

The evidence that vitamin D is valuable for the teeth is based firstly on animal experiments and secondly on human investigations The animal experiments were chiefly done by Lady Mellanby on dogs [270] She showed that the teeth of rachitic puppies had poor thin enamel and the dentine was what she called hypoplastic These teeth did not decay, since dogs have a naturally immune saliva (p 570) One also wonders whether part of their poor structure was due to the puppies never chewing or biting anything hard "Function creates structure," and in these puppies the teeth had no function—the food was pap they were too ill from their rickets to gnaw their cages as did the healthy control puppies and when they were allowed outdoors they were muzzled

However, having shown that rachitic puppies have poor teeth which do not decay, the next step was to investigate the shed milk teeth of children which had decayed Those teeth which had most caries were found to have the poorest structure reminiscent of the rachitic puppies' teeth This was held to show that the teeth decayed because they were bad and bad because they were rachitic This argument only contains two possible fallacies firstly bad teeth may be associated with decay because chronic bad health —from whatever cause—might hinder both the proper formation of the teeth and the production of immune saliva, secondly, there was no reason to believe the children who had bad teeth were rachitic [271] except that they had bad teeth—in fact the argument having gone in a circle gets nowhere

The clinical value of vitamin D was tested by giving it as a supplement to groups of children and comparing their teeth to those of children who had had no such supplement Many investigations [271] both on a large and a small scale in England and America showed that by increasing the amount of vitamin D in the diet not only was the number of teeth which subsequently developed caries reduced, but that caries which had started tended to be arrested It must, however, be noted that full protection was not given Thus in one investigation over a period of about eight months the children receiving "abundant fat soluble vitamins" had on an average 1 4 teeth showing fresh or increased caries against 5 1 teeth in children who had little vitamin and some oatmeal So that though the teeth were far better preserved with extra vitamin D yet the degree of caries was still deplorably high McBeath and Zucker [177] found the natural vitamin more valuable than the synthetic

The arguments against vitamin D being directly responsible for this improvement are —

(a) Some investigators have not found that vitamin D has any effect, even on recently erupted teeth, when given for over a year to large groups of children [272]

(b) Fish [273] found experimentally that there was no evidence that vitamin D had any effect on formed dentine or enamel, so that it is difficult

to understand how the enamel, and so the resistance to decay, could be improved by vitamin D

(*c*) The faults in the rachitic teeth of puppies are not like the faults where caries starts in human teeth [263]

(*d*) The teeth which should show most hypoplasia, if this is due to lack of vitamin D, are those which are formed during the first year and a half of life when rickets is most common The incisors should thus be affected most, they are affected least Then the most affected in order are the second molars, first molars, canines, though they erupt in the order of first molars, canines, second molars [274, 275] Thus there is no relation between hypoplasia and the teeth formed at the period when vitamin D tends to be most deficient

(*e*) Children with extensive progressive caries do not commonly show any signs of rickets [271], and children with severe rickets, even when born of osteomalacic mothers may have no caries (p 570)

(*f*) Dental caries in rats is not prevented by vitamin D [278]

We are then, left with the fact that vitamin D decreases caries to some extent in some children, but that caries is not dependent on a deficiency acting directly on the teeth themselves It appears most likely that vitamin D improves caries only indirectly by improving the general health and nutrition of the child—both factors which have an effect on caries [218, 219, 264, 276]

Immunity to dental decay depends on eating coarse unrefined food—especially unrefined sugar and stone ground flour—raw milk and a good diet There is no simple specific against decay such as vitamin D, and cleaning the teeth cannot compensate for a bad diet.

The absolute necessity for vitamin A at ages when the enamel is being formed, and the importance of vitamins A and C for the health of the gums, and so indirectly for the preservation or rather fixation of the teeth, must be remembered

Dental Decay and Pregnancy. The old saying that "every child costs a tooth" appears to be only explicable on the spread of paradontal disease during pregnancy Actual dental decay during pregnancy does not appear to increase even under the added strain of osteomalacia (p 570) Fish [277] states "There is no active evidence as yet of any definite loss of calcium *from the dentine under any circumstances,"* *including pregnancy, low* calcium diets, excessive vitamin D, and parathormone injections Protection during pregnancy depends on the dietetic factors already mentioned, on cleaning the teeth, and not simply on taking more vitamin D

TUBERCULOSIS

Lupus Vulgaris In May, 1848 Devergie [279] described the treatment of lupus vulgaris with large doses of cod liver oil—the "l'huile brune" variety, because it was more fishy In September of the same year Emery [280] also advocated cod-liver oil, increasing the dose to about two pints daily Though he gives detailed instructions as to how to overcome vomiting, etc, and [illegible] with improve- [illegible] probably lying [illegible] only one third [illegible] impossible as a dose Devergie states that "Aujourdhui je n'hésite pas à declarer que c'est de tous le plus efficace je vois plus loin, et j'avance qu'administré seul il guerit Cette assertion est tellement vraie, qu'à partir du moment ou ce moyen a ête mis en usage par quelques-unes de nos malades, les autres m'ont successivement prié de le leur faire prendre"

The oil had to be taken for three to six months or longer He started with "une cuilleree à bouche matin et soir" Every two or three days he increased

the dose by another spoonful After the dose had been increased to two to three spoonfuls at one time the patients overcame their first nausea, and then the dose could rapidly be increased to the twelve to fourteen spoonfuls daily which he found were necessary The patients felt very well, their appetite went down and their weight up

He had been using this treatment for eight years He gives no figures of the numbers of patients who recover, but from his previous accounts of other treatments and his statement that general and local treatment must be combined, it seems probable that he was not getting a very large proportion of cures

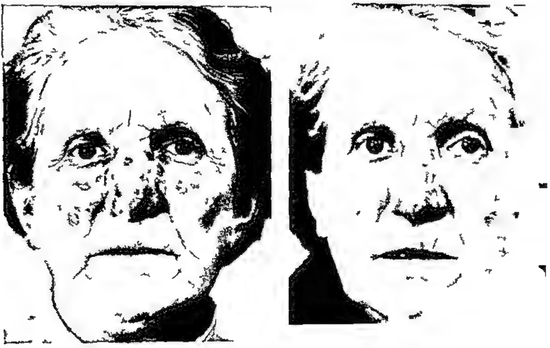
Until the Second World War this French treatment, like all French work of the last century on vitamin D, was completely forgotten Then Dowling and Prosser Thomas [281] in England, Famelle [282] in Belgium and Charpy [284] in France all independently reported that calciferol in large doses was a highly successful treatment for lupus vulgaris In Belgium such treatment is called after Famelle while on the rest of the Continent it is called after Charpy The value of calciferol has now been confirmed in nearly every country in the world

Fig 202 One of the first cases of lupus vulgaris treated in England with calciferol extensive lesions of the face and neck almost completely healed after 150 000 I U calciferol daily, later reduced to 100 000 I U, for one year

The dose of vitamin D used in England for adults is 150,000 I U daily given by mouth as tablets, 100,000 I U. is less certain in its effects and 50,000 I U is useless [282] An ineffective dose remains ineffective however long it is continued [282] For children the dose has generally been 100,000 I U daily [287] There is no certainty as to whether treatment should be given continuously, possibly for a year or more, until a clinical cure is achieved or whether short courses each of two or three months are equally good and less dangerous In England treatment is stopped when there is a clinical cure If there is a relapse it is better to wait until the condition has developed, rather than to restart treatment immediately [287] Local treatment in the form of Finsen therapy, caustics, etc, must also be used [284, 287] On the Continent the general custom is to give the Famelle or Charpy form of treatment, that is 600,000 I U twice weekly by mouth for a month, followed by the same dose weekly for two years, irrespective of clinical cure [285, 286] Calcium or milk is also given and a diet rich in everything except fat and salt. However, workers in Scandinavia [300] Holland [290] and Denmark [289] have reported excellent results on doses similar to those used in England and without any extra calcium or special diet Though emphasis has been

laid by Charpy on giving calciferol dissolved in propylene glycol [285] results are just as good when the solvent is oil [289] or no solvent at all is used but dry tablets instead as in England Lightbound [294] claims that giving calciferol by intramuscular injection reduces its toxicity and gives a more rapid cure while Moguer [292] found that infiltrating the lesions with weekly injections of 100 000 I U brought about a cure in seven to nine weeks In resistant cases injections twice weekly of 500 mg of unirradiated ergosterol in oil [293] or 50 000 I U of vitamin A by mouth daily [291] have been said to be of value though ergosterol in enormous oral doses is valueless [242] Feeny [243] has given an excellent account of the use of calciferol combined with chemotherapy

Improvement may occur in a couple of weeks and a clinical cure in two months though often it is delayed for over a year A Herxheimer like reaction is frequently present in the first few weeks of treatment with an



A B

FIG 203 Lupus vulgaris before and after treatment for eight months with 100 000 I U daily of calciferol

increase in the redness and swelling of the lesions and even ulceration [287] As in the first English case ever treated [281 282] great improvement may be caused by a violent toxic reaction [287] though many patients are cured without any toxic symptoms Such symptoms and the biochemical effects of calciferol are discussed on p 578

Microscopical examination of the lesions shows that under the influence of calciferol a quantity of young connective tissue develops about the giant cell systems which are invaded and disintegrated so that in the end there is only an area of fibrosis and lymphocytic infiltration [287] before this the histological picture resembles sarcoid [286 287] However Ruiter and Groen [290] found that of fifteen clinically healed cases six had microscopic lesions from one of which tubercle bacilli were grown and tubercle bacilli were also grown from another three cases which had no microscopic lesions Leuggenhager and others [295] even report that three quarters of their clinically cured cases still showed microscopic infection Healed lupus skin contains more calcium than normal skin from the same patient [299]

Clinical cures vary from virtually 100 per cent at the end of treatment

[285 287 289 300] to only 30 per cent [290] Relapses are less common than would be expected from the persistence of infection in cured cases ranging from none [285] to about one quarter [287 300] Lesions of mucous membranes respond excellently [284 300]

The reason why calciferol acts is obscure it is not due to elevation of the total serum calcium since this may remain virtually normal in cases which are cured [282 287] though Anning and others [288] have from a small number of cases suggested the possibility that the rise in the diffusible calcium is important Charpy [285] believes that mobilization of phosphorus is the key action which is congruous with the action of vitamin D (p 535) tubercle bacilli themselves [290] are not sensitive to calciferol since they have been grown in Dubos liquid medium containing 10 to 1 000 I U per 100 ml from lesions which both did and did not respond to treatment Further human and bovine tubercle bacilli are equally insensitive to calciferol [290] nor does this have any greater inhibiting effect in solid media [287]

Scrofuloderma [296 297 308] may be virtually cured or may flare up [296] while the results are only moderately good in *erythema induratum* [284 297] the condition sometimes being made worse [296 308] *Papulonecrotic tuberculide* [297] is said not to be affected

The contraindications to the use of calciferol in large doses are discussed on p 579

Other Forms of Tuberculosis Calciferol used in the same doses as for lupus vulgaris is excellent for tuberculous adenitis both in children and adults causing a rapid healing of sinuses and a shrinking and calcification of the glands which leaves them mobile and easy to excise [283 284 287] Tuberculous peritonitis [287 301] cystitis [287 301] and epididymitis [284] have not been extensively treated though cases have responded in a miraculous manner [287 301] while infections of bones and joints [287] may be benefited Pulmonary tuberculosis may be made worse or better (p 579)

Boeck s Sarcoidosis was not benefited in eight cases compared to eight control cases by Nelson [302] and Pascher and his colleagues [308] only mildly improved one of seven cases but single cases have responded [303 304 305] especially when there was a toxic reaction to calciferol and Curtis and others [306] have added another six cases of improvement It is however the skin which improves the lesions in the lungs and bones remaining unaffected or becoming worse [305]

Leprosy though so akin to tuberculosis does not react in the same dramatic manner to calciferol Floch and Destombes [307] after the treatment of twenty one cases only being able to say that further work is worth while while De Castro and Haedo [297] obtained no results in six cases

FURTHER CLINICAL USES OF VITAMIN D

Endocrine Disorders *Cretinism* Cretins when they start taking thyroid often grow amazingly fast Rickets as a result may occur so the possibility must be kept in mind and ample cod liver oil given as a prophylactic measure

Thyrotoxicosis The action of vitamin D in very large doses on the thyroid has been discussed on p 533 The possible value of cod liver oil as far as its vitamins are concerned is probably due to its vitamin A

Parathyroprivic Tetany The use of vitamin D in controlling the tetany

since it raises the

It also stabilizes

less tendency to

sudden tetany [111] but as the effect of vitamin D in relieving tetany takes several days it is useless in post operative emergencies In prolonged

treatment it has the great advantage of not losing its effect while parathormone on the other hand does so after repeated administration

There is the danger that large doses of vitamin D may have toxic effects but by using the natural concentrated fish liver preparations by keeping the dose at the lowest level and stopping it for short periods where possible harmful effects are unlikely to occur (p 578)

The dose of vitamin D must depend entirely on the individual case With a diet high in calcium and low in phosphorus 20 000 units should be given daily by mouth and the amount adjusted from the clinical results Severinghaus and St John [112] however state that the diet need not be carefully controlled since they have successfully treated six women who ate as much meat milk and eggs as they liked Four of the women were observed for over two years and one of them had an uneventful pregnancy Vitamin D combined with calcium salts was given orally in doses of 150 000 to 400 000 I U daily the correct amount being adjusted partly by blood calcium estimations and partly by subjective symptoms of which tingling in the arms and hands was the earliest and most reliable indication for increasing the dose Implants of vitamin D tablets have been advocated [309] to our minds most unwisely since the amount absorbed cannot be adjusted to meet the needs of the individual patient

Dihydrotachysterol one of the products of the prolonged irradiation and hydrogenation of ergosterol called commercially A T 10 (anti tetanic preparation [illegible] D for the treatment of tetany by [illegible]] and many others It has no ap[illegible] the blood calcium more rapidly than vitamin D It is however more toxic and in some cases has to be given in increasing amounts It also has a more erratic effect on the level of the calcium in the blood It appears to have no advantages and severe drawbacks compared to vitamin D apart from its more rapid action in raising the blood calcium

Sexual Disorders An occasional increase in libido of both sexes and also in sexual capacity and an improvement in the rhythm of the menses has been reported in a small proportion of cases who were taking very large doses of vitamin D [111] The therapeutic use of vitamin D for sexual disorders however is ruled out by the dangerously high amounts which must be given and by the small proportion of patients who will benefit

The Bleeding Tendency in Jaundice and Hepatic Insufficiency It is very surprising that vitamin D is seldom used for preventing the hæmorrhage which is so common and so disastrous in patients with jaundice or hepatic insufficiency who have to undergo an operation In a very large series of carefully controlled cases Gray and Ivy [117] and McNealy and others [118] found that when the Ivy bleeding time was prolonged the operative outlook was bad because of hæmorrhage during or after the operation But vitamin D given thrice daily by mouth in doses of about 7 000 I U for four to fourteen days before operation almost invariably reduced the bleeding time to normal and prevented any hæmorrhagic complications When there was no bile in the stools bile was given by mouth to aid the absorption of the vitamin W[illegible] profound vitamin D did not improv[illegible] e a hæmorrhagic tendency in any [illegible] Why vitamin D only has an effect in one kind of hæmorrhage is obscure it does not act through changing the blood calcium [117] or through correcting the prothrombin deficiency [120] which is often found in jaundice owing to poor absorption of vitamin K

Lead, Cadmium and Radium Poisoning Lead behaves like calcium from the point of view of storage in the body [65 104 222 223 313] During the ac[illegible] the ionization of [illegible] 300 I U

of vitamin D a day should be given to aid the deposition of the lead in the bones Calcium lactate 180 grains daily should be given at the same time both to decrease the ionization of the lead and also to ensure that lead and calcium are being deposited and not mobilized in the bones

After the acute symptoms of lead poisoning are over it is necessary to de lead the bones Vitamin D in large doses is not so certain in its results as parathormone and both need careful supervision Ammonium chloride must be used when patients cannot be carefully guarded against excessive mobilization of lead bringing back the acute symptoms of poisoning In any case the diet should give a low calcium high phosphorus intake—the latter to depress the ionization of the lead in the blood [65 104] Cadmium poisoning [69] on the other hand only needs to be treated like osteomalacia (p 569) being really the same disease

Radium is so toxic that it must be eliminated from the body as quickly as possible vitamin D being of less value than the other measures mentioned for de leading [104]

The Healing of Fractures About 600 I U daily of vitamin D should be given to patients with fractures This ensures that the calcification of the callus shall not be prevented by lack of vitamin D which is especially important in the old [108 211] where mild osteoporosis is common Massive doses of vitamin D delay calcification [314] A T 10 (p 576) has also been extensively and to our mind unwisely used to hasten calcification Irradiation has also been suggested [315] The use of ascorbic acid in the healing of fractures is discussed on p 407

Arthritis Reed and his collaborators [111] sum up the effect of using vitamin D in arthritis thus A group of forty five cases of atrophic arthritis seven cases of hypertrophic arthritis and three mixed cases were treated with 150 000 200 000 units of vitamin D daily with definite measurable improvement in 74 5 per cent of cases as manifested by reduced swelling and pain increased mobility (active and passive) improved muscular tonus and weight gain The authors point out that there is a definite risk from such large doses though apparently less than one per cent of cases showed toxic symptoms Small doses should be given at first to avoid risk in unduly susceptible patients (p 5-8) Treatment has to be continued for many months or even years No claim is made for any physiological reason for this form of treatment Good results have also been reported by other workers [316 317 318] But Freyberg [319] from a most careful study in which he used the reputedly pure activated vitamin D reports that six out of thirty six patients had to give up treatment because of toxic symptoms and that this form of therapy should not be relied on as the only form of treatment for seldom is the course of the disease favourably altered The general impression is left that other forms of treatment over such a long period would have as good results without the very real danger of toxic damage

Skin Diseases Tuberculous infections of the skin sarcoidosis and leprosy have been discussed on pp 5-2 to 575 Many other dermatological diseases have been treated with large or toxic doses of vitamin D probably it is useless in all of them though there are unsatisfactory and unconfirmed reports that it has occasionally benefited scleroderma [320 321] acne [321 322] leukonychia totalis and pemphigus [321] eczema and epidermolysis bullosa [323] granuloma annulare which may on the other hand be made worse [308] and lichen planus [308] Chilblains are neither prevented [325] nor cured [284] and in our experience cod liver oil—discussed fully on p 533—and vitamin D ointments have been valueless in diabetic ulcers bed sores and varicose ulcers

Psoriasis has been very fully investigated but again it would appear that treatment with vitamin D is quite ineffective In the most thorough investigation yet carried out which was reported by Clarke [324] and carried

out by twenty two doctors on one hundred and forty one patients only twelve per cent improved spontaneous improvement should have given a better figure Kindler [326] reviewed the literature in 1949 and from his own treatment of thirty one cases of whom twelve were virtually cured believes vitamin D is on the whole worth trying while Wright [323] after treating forty five cases believes it is useless and Madden [327] from his experience with twenty four patients states that improvement is caused only when the patients are made toxic by vitamin D, when it is stopped the psoriasis recurs

VITAMIN D POISONING

All preparations of synthetic vitamin D_2 however pure they are reputed to be are toxic for man in large doses [288] and so are synthetic vitamin D_3 [342] and concentrated fish liver oils two children dying from drinking large quantities of cod liver oil while also being exposed for long periods to the sun

The dose of vitamin D which when repeated daily is toxic varies greatly with the individual From the great numbers of patients with lupus vulgaris (p 572) who have received 100 000 to 150 000 I U daily or 600 000 I U weekly it is definite that such doses are always on the verge of toxicity and in about twenty per cent of cases cause definite toxic symptoms and in a larger percentage of cases abnormally high levels of total serum calcium or of diffusible serum calcium [288] Adult cases have been reported in whom only 25 000 I U daily of a very pure activated preparation of vitamin D have caused toxic symptoms [319] Reports seldom mention how soon toxic symptoms may appear but on daily doses of 700 000 I U they have occurred in ten days [12J] and the same time appears to have elapsed in some of Anning and his collaborators patients [288] Toxicity however may not appear for about a year and after a very large total intake such as 35 million I U Toxicity is said to be precipitated by emotional strain indigestion and constipation [111] though the last two conditions may really be the result and not the cause of the toxicity

Children may be given single very massive doses with no obvious ill effects (p 545) but repeated large doses may be disastrous Debré [328] in 1948 saw or collected from the literature thirty one children who developed acute poisoning of whom twelve died Delayed toxic symptoms may be and doubtless generally are completely overlooked since doses of 1 800 I U daily in infants only cause slight retarding of growth and delayed dentition (p 532) Larger doses may however have most serious delayed effects Thus Briskas and Maret [329] saw one infant of eight and a half months and two children of ten years whose growth appeared to have been severely perverted in different ways depending on the age when the excessive vitamin D was given The infant had received 4 million I U in the preceding two months sixteen teeth were present the centres of ossification were those of a child of eighteen months growth was excessive with wasting and hypotonia One of the children had received 20 million I U over several years he was pale thin and very hypotonic with the appearance of a boy of four or five and carpal centres of ossification of a boy of five and a half his liver was not enlarged and his blood calcium and phosphorus were normal The other boy of ten had had 10 million I U over three months when he was seven years old he was mentally retarded very thin and profoundly hypotonic his height and centres of ossification were normal Though the evidence is obviously dubious that excessive vitamin D was responsible for the condition of these children yet they have been described so fully to emphasize that the serious effects of poisoning may be covert and delayed

The method of administration alters the toxicity the more rapidly vitamin D is absorbed the more toxic it becomes Thus when given by mouth in oil it is said to be less toxic than when given in alcoholic solution [330]

and at least in dogs rapidly absorbed injections are highly toxic while oily injections are not [128] which appears to be broadly true in man as regards the relative degree of toxicity of different types of injections [128 294]

Indications for Using Large Doses of Vitamin D with Caution Fat patients as their fat is largely inert from the point of view of metabolism should not be given very large doses to begin with Patients confined in bed may also metabolize vitamin D slowly since two with fractures rapidly developed serious toxic symptoms and the healing of the fractures was delayed [331] while the risk in young patients of renal calculi is severe (p 563)

Personal sensitiveness is important and can be avoided only by beginning with small doses One patient has been reported who showed toxic symptoms on 25 000 I U daily [319] and one infant who was frequently outdoors in the sun died from taking a concentrated cod liver oil which only gave him about 1 500 I U daily [179]

Nephritis and cardiovascular degeneration are contraindications for the use of high doses of vitamin D since both may be exacerbated Two elderly men with such conditions have been reported by Steck and others [129] as dying from poisoning with vitamin D

Children require watching One child we saw in hospital died from both being exposed to the sun and also *drinking not only his own cod liver oil* but also that of several other children in his ward

Pulmonary tuberculosis is a reason for giving large doses with the greatest caution since Ridderbos [334] made all of nine severely ill patients worse and eighteen of fifty six patients who had less extensive pulmonary lesions Other workers [286 296] have also reported bad effects though when used with chemotherapy it may be of value possibly by causing a local vascular reaction which enables chemotherapy to reach the infection [344]

Symptoms of Poisoning The symptoms of chronic poisoning in children have been discussed above In acute poisoning that is in poisoning which produces overt symptoms during treatment the adult patient may suffer in a great variety of ways almost all of which have been collected with references to the relevant papers by Anning and others [288] General well being and a good appetite is often the first symptom of poisoning In this lies a danger for doctors who buy large bottles of concentrated irradiated products for dispensing and drink them often in huge doses for the tonic effect that they give

Loss of appetite follows quickly on the sense of well being and loss of weight greater than would be expected from the loss of appetite After this intestinal symptoms such as nausea vomiting constipation or diarrhœa increase rapidly Abdominal pain may be so severe as to inveigle surgeons into performing laparotomies

Thirst may be intense and the urine is increased and voided frequently by night and day Shortly before death dehydration occurs with scanty urine

Weariness and weakness and more rarely profound mental depression come on and may be early symptoms The memory occasionally is confused Restless excitability may also occur and photophobia and dislike of noise

Headaches are usual and one special form has been noticed which may be the first symptom to arouse suspicion This is a tightness across the back of the head which goes on to acute sensitiveness of the scalp so that the patient cannot rest his head on the pillow The same tenderness at the back of the head has been found in dogs Pain along the jaws and tender teeth have been noticed and pains in joints and muscles Profuse sweating may occur The hands and feet occasionally feel numb and tingle, and polyneuritis [332] has been reported Epileptiform fits are a rare complication which cease when treatment is stopped [333]

Aphrodisiac effects to the extent of social inconvenience have been occasionally noted without other toxic symptoms

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FIG. 204 Multilocular calcifications of hip in a man of fifty six who had taken 150 000 I U of vitamin D daily for two and a half years

FIG. 205 The same as Fig 204 showing resolution of calcification fifteen months after vitamin D was stopped

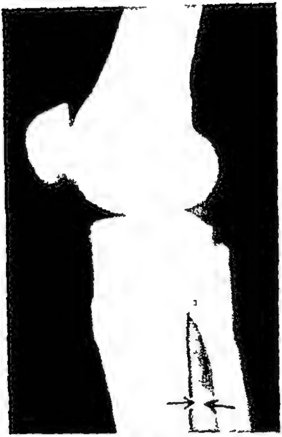

Fig. 206 The same patient as in Fig. 204 showing calcification of posterior tibial artery

Fig. 207 The same patient as in Fig. 204, showing calcification of the abdominal aorta

In fatal cases the general picture is that of uraemia which indeed it generally is

Th[illegible]

mer[illegible] sudden insistent and persistent vomiting may occur and thirst and polyuria may be extreme, a child of two passing as much as six pints a day Intense constipation is common interrupted by brief attacks of diarrhoea Pain may be absent or may be occipital or frontal or in the abdomen joints or muscles

FIG 208 The same patient as in Fig 204 showing calcification in the soft tissues of the wrist inter phalangeal joint and tip of the ring finger

The child is very thin wan and sallow with a dry pale skin though he is seldom anaemic His blood pressure is raised From being irritable and depressed he slips into stupor without meningeal signs or stiffness and with a normal cerebrospinal fluid He lies dying curled up on his side, his breathing heavy and deep his pulse perhaps slow, his temperature normal or high Sometimes generalized convulsions interrupt the stupor [179 328 340]

Biochemical and Pathological Changes in Poisoning The urine is loaded with calcium and phosphorus and may contain *calcium* casts if it is not too acid It is said that Sulkowitch's reagent may show this excessive calcium excretion even when the total serum calcium is normal and so gives a valuable warning of impending toxicity (p 563) as does the low specific gravity in the *morning* Albumin occasionally and sugar and blood rarely are found in the urine Renal function is generally impaired and the blood urea rises [288]

The total serum calcium need not be raised though the patient is frankly toxic or conversely it may be very high and the patient in the best of health [282 287 328] Anning and his co workers [288] found that the diffusible calcium was definitely raised in all their thirty toxic cases 7 5 mg per 100 ml being taken as a warning that the patient was verging on the toxic especially if there was no parallel rise in the total serum calcium The inorganic serum phosphorus is not constantly affected and the alkaline phosphatase remains normal [288 328] The plasma protein content is increased [288] which may be the cause of the increased erythrocyte sedimentation rate The latter rises at the beginning of the treatment of lupus vulgaris and then falls unless

toxicity supervenes when it rises again [287] The blood pressure and electrocardiogram remain normal [288]

X rays may show calcification of the larger arteries [288, 335] and in children of the lungs and soft tissues [330] There may be generalized osteoporosis and when this is accompanied by large smoothly lobulated masses of metastatic calcification, confined to periarticular structures, the diagnosis lies between *vitamin D poisoning, chronic nephritis* and *hyperparathyroidism* [337] In children there is increased density of the zone of provisional calcification with an area of rarefaction proximal to this and also periosteal thickening with a dense outer layer and a rarefied inner zone [336]. *Metatastic calcification visible to the naked eye* [338] or by a slit lamp [328,

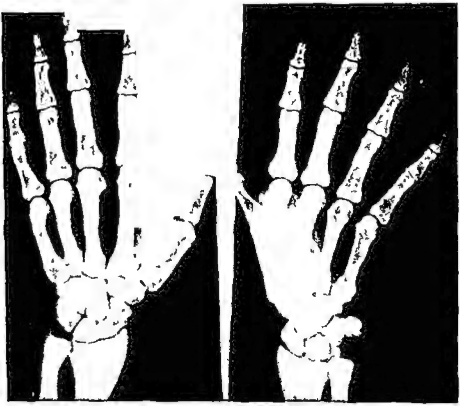

FIG 209 The same hand as in Fig 208 fifteen months after stopping vitamin D All the deposits of calcium have been absorbed

339] *may be seen in the sclera, cornea and conjunctiva* : it appears as small glass like particles beneath the conjunctival basement membrane.

Post-mortem examinations [288] of fatal cases show almost constantly calcification of the renal tubules and less constantly general calcification of the kidneys, of the larger and smaller arteries, of the myocardium, of the [illegible]

a few days, but in severe cases, especially in infants, intravenous normal saline should be given, since the cause of death is the loss from the body of its electrolytes [327] This loss is due to the diuresis caused by the kidney's efforts to secrete the excessive calcium and phosphorus

Prognosis. The outlook is good As has been said on p 534 the damage to the tissues and even the calcification disappears in animals and may in man [335] (Figs 205, 209) In man there is no evidence as to the ultimate

effect of prolonged overdosage. No bad chronic effects have been [illegible]

[illegible] In children who have recovered from convulsions, the prognosis must be guarded as there may be a relapse after several months [328] or later impairment of development (p. 578).

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CHAPTER VIII

VITAMIN E

THE ANTISTERILITY OR ANTIDYSTROPHIC VITAMIN ALPHA BETA GAMMA OR DELTA TOCOPHEROL

Vitamin E is the name generally used when speaking of the vitamin in general or as it occurs in foods Alpha tocopherol is the most biologically active of several very similar substances all of which have the properties of vitamin E Thus while alpha tocopherol means one distinct substance vitamin E may mean either alpha tocopherol or a mixture of this and other similar substances To avoid confusion vitamin E should be only used in the latter sense and not as a synonym for alpha tocopherol The name tocopherol is derived from the Greek *τοκος* childbirth and *φερω* to bear

HISTORY

Herbert McLean Evans of California will always have his name associated with vitamin E partly because he and Bishop [1] in 1922 demonstrated the existence of an antisterility vitamin and partly because of the monograph on vitamin E written by himself and Burr [2] in 1927 This work remains to the present day the foundation of our knowledge of vitamin E The authors showed that the foods richest in the vitamin were green leaves and the germ of seeds Wheat germ and wheat germ oil were found to have a remarkably high content of vitamin E and remain to this day the best source Rats were the experimental animals used and for these animals it was proved that a deficiency of vitamin E leads to sterility in the male and abortion though not failure to conceive in the female

The existence of the vitamin however had been foreshadowed in 1920 by Mattill and Conklin and confirmed in 1922 by Mattill and independently in 1923 by Sure This early work is summarized by Evans and Burr [2]

Until 1928 vitamin E was thought to be entirely concerned with reproduction but in this year Evans and Burr [3] reported that young rats suckled by vitamin E deficient mothers became paralysed while Goettsch and Pappenheimer [4] in 1931 showed that guinea pigs and rabbits when deprived of vitamin E developed a primary muscular dystrophy histologically identical with the progressive muscular dystrophies of man From Denmark Ringsted [5] in 1935 and Einarson and Ringsted [6] in 1938 published careful and extensive research on the effects of lack of vitamin E on the central nervous system of adult rats They pointed out that the neurological degenerations which were produced resembled those of amyotrophic lateral sclerosis and tabes dorsalis in man

It is a depressing demonstration of the lack of co ordination between research workers and clinicians that it was not until nine years after the discovery of vitamin E that Vogt Moller [7] in Denmark first put it to any useful purpose by treating sterility in cows In the same year he treated two women with habitual abortion with success [8] and six years later Young [13] in England and Shute [14] in Canada reported good results in the treatment of threatened abortion and pregnancy toxaemias

Bicknell [9] in 1938 seven years after the possible value of vitamin E in human muscular dystrophy had been implied by animal research started to treat cases of muscular dystrophy and neurological degeneration with vitamin E or rather wheat germ The improvement in his cases was

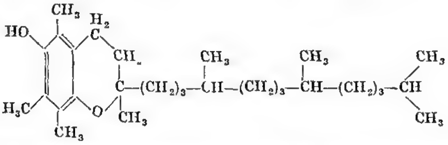
reported in 1940 Stone [10] and Wechsler [11] in 1940 reported cases of muscular dystrophy and amyotrophic lateral sclerosis successfully treated with vitamin E, but it was only in 1941 that the subject began to arouse wide clinical interest The early promise, however, of the value of vitamin E in the treatment of muscular and nervous diseases has not been confirmed by later work, which at best only suggests that these diseases are caused by some complicated failure in the metabolism of the vitamin, which can only be corrected in rare cases by simple treatment with the vitamin alone Many other conditions—especially those due to cardiovascular degenerations —have also been treated, but the results have been disappointing Indeed, the clinical value of vitamin E is still obscure in spite of the great volume of experimental work during the last ten years, which shows how essential is the vitamin for many physiological processes, probably acting both in a non specific manner as the body's chief antioxidant and also specifically in some enzyme systems

The elucidation of the chemical structure of vitamin E was rapid after 1936 In this year Evans, Emerson and Emerson [12] isolated from wheat germ oil two alcohols, alpha- and beta tocopherols, and from cotton seed oil gamma-tocopherol, all of which had vitamin E activity

Further work by many investigators, including Todd, Bergel and Drummond and their collaborators in England, Fernholz in America, and Karrer and John on the Continent, finally led to the synthesis of alpha , beta- and gamma tocopherols and the elucidation of their chemical structure [7], though only in 1946 were the existence and the properties of delta-tocopherol discovered by Stern, Robeson, Weisler and Baxter [15]

CHEMISTRY OF VITAMIN E

Vitamin E occurs as alpha-, beta , gamma and delta-tocopherol, and there are also various synthetic compounds which may [7, 16, 17] or may not [208] possess some slight activity Alpha tocopheryl hydroquinone and alpha tocopherylquinone, the first oxidation products of alpha tocopherol, are said to prevent dystrophy in the rabbit [18, 206], but not fœtal resorption in the rat [22] Indeed, in the mouse [207] fœtal resorption is actually caused The chemistry and the physical properties of the tocopherols is extremely complex , the papers by T [illegible] and Stern, Robeson and others [15, 20, 21, 41, [illegible] should be consulted for discussions on the subj [illegible] copherol is



Beta and gamma tocopherols are isomers, identical with alpha tocopherol except that they possess two methyl groups in the aromatic nucleus, while in delta tocopherol there is only one [15]

The tocopherols are oils at room temperatures giving maximum absorption at 290 to 294 millimicrons They are soluble in all lipoid solvents and the phosphate is moderately soluble in water, which is the reason why it is used in preference to the other esters, which are insoluble in water, for studying the effect of vitamin E on enzyme systems The tocopherols are stable to strong alkali and to strong hydrochloric and sulphuric acids, but they are very easily oxidized, which is the basis of chemical methods of

estimation and is also the reason why they are such important biological antioxidants for fats and why *they are so rapidly and disastrously destroyed by rancid fats* (p 615) They are unstable in air and when exposed to ultraviolet light, though their esters are not The stability of vitamin E in foods is discussed on p 627, and the great differences in biological activity between the various tocopherols and also their esters are discussed below

UNITS OF VITAMIN E

The International Unit of vitamin E is the amount which, when administered orally, has the same specific activity as 1 mg of synthetic racemic alpha tocopheryl acetate in preventing gestation resorption in rats deprived of vitamin E

The different forms of alpha toc[illegible] oxidant potencies Judging by the [illegible] the following are the values in I U

Synthetic racemic alpha tocopheryl acetate	1 00
,, ,, ,, tocopherol	0 68
Natural dextrorotatory alpha tocopheryl acetate	1 36
,, ,, ,, , succinate	1 21
,, ,, ,, tocopherol	0 92

The greater value of the esters is probably due to their greater stability in the gut before absorption, but the allophanates have no activity [19, 210 211]

The four tocopherols vary greatly in their activities *For preventing* gestation resorption in rats the relative activities of alpha, beta, gamma [210] and delta [15] tocopherols are 100 40 8 3 and 1, while comparing the first three racemic synthetic tocopherols to each other [27] values are 100, 25 and 19 Judging by the cure of muscular dystrophy in rabbits [212] values for the first three natural tocopherols are 100, 30 and 20 and for synthetic alpha and gamma tocopherols the values are eighty two and thirty per cent of their natural forms Judging by resistance to hæmolysis [226] the relative potencies of the four natural tocopherols compared to racemic alpha tocopherol are 1 33, 0 17 0 07 and 0 04 Storage of the tocopherols in the body and their transfer by the hen to the egg are discussed on p 600

For a long time the *in vitro* antioxidant potencies of the different tocopherols appeared to be exactly the opposite of their different biological potencies *which was difficult to harmonize with the belief that the tocopherols* owe their biological activity to their antioxidant properties But the excellent review and work of Kunkel [213] in 1951 discusses and solves this difficulty

stored to a far larger extent in the body (p 603) Alpha tocop[illegible] said to be as potent as alpha tocopherol in the cure of muscular dystrophy [209] Other substances which may have vitamin E potency are referred to on p 593

FUNDAMENTAL OR BIOCHEMICAL ACTION OF VITAMIN E

Vitamin E probably acts as a non specific general antioxidant in all the tissues of the body It may also possibly act as part of a specific enzyme [illegible] as yet no clear proof If all its action is merely

easily answered by postulates which are largely [illegible] firstly, that different amounts of vitamin E are stored in or are made available

o different tissues so that some are more protected than others secondly that the metabolism of different tissues differs in its need for antioxidants nvestigating the action of vitamin E is difficult partly because the bio hemical changes which occur in the living deprived animal are only the end esult of an already far advanced biochemical chaos and partly because the ddition of vitamin E to tissues or isolated enzyme systems does not eproduce its action in the intact animal In the following discussion the biochemical action of vitamin E will be considered as it appears firstly from tudies on living animals secondly from studies on isolated tissues and hirdly from studies on isolated systems

The living Animal The antioxidant activity of vitamin E starts within he lumen of the gut where it protects vitamin A and carotene (p 23) and biotin and the vitamin B complex [184 185] against destruction by rancid insaturated fatty acids This protection for vitamin A is continued within he body (p 23) though apparently it is not necessary for the preservation of biotin in the tissues [186] Vitamin E is the only antioxidant present in body fats [195] and it probably plays the same role in fish liver oils [196 284]

Oxygen consumption greatly increases in the young rat deprived of itamin E before there are any signs of muscular dystrophy [187] When nuscular dystrophy appears the oxygen consumption falls in rats [187] and emains normal in guinea pigs [188] but probably this is only because partial nanition counteracts the effect of lack of vitamin E The increase in oxygen onsumption in the intact animal can be wholly accounted for by the ncreased oxygen consumption of the dystrophic muscles [117 187 189] and o does not necessarily involve an increase in any other tissues which in he liver at least does not occur [187] Injections of alpha tocopheryl phosphate reduce the oxygen consumption of muscles removed at biopsy almost to normal within four hours though the same effect is only achieved by oral doses of alpha tocopherol in twenty seven hours [117] In normal children vitamin E decreases the basal metabolic rate and the specific dynamic action of glycine [190]

Phorphorus turnover in all the tissues of the body is stimulated by lack of vitamin E This is thought by Weissberger and Harris [191] not to be a direct effect of the deficiency but a secondary result of the increased oxidation n muscle since here oxidation and phosphorylation are coupled so that what affects one will affect the other It is considered that this increased urnover in muscle leads to the increased turnover which occurs in both exes equally *in the bone kidney blood uterus ovary testicle and muscle* 191] It is of profound interest that no greater change in phosphorus metabolism occurs in dystrophic muscle than in bone or blood and also that he change in the genital tissues—which in the rat are the first to be damaged by a deficiency—is no greater than that in other tissues Doses of vitamin E of at least one hundred times the normal requirements also cause an increase n the turnover of phosphorus but this is not similar to that caused by a deficiency since it is greater it occurs more rapidly and it is never associated with pathological changes in muscle though on the other hand osseous arefaction occurs [191]

Creatine is decreased in dystrophic muscle [117 131 182] and is excreted n large amounts in the urine even before there are any clinical signs of a deficiency of vitamin E [29] Again this abnormal creatine metabolism may be held to be a secondary effect of the uncontrolled and excessive oxygen and phosphorus metabolism of the muscle Alpha tocopheryl phosphate injections reduce still further the creatine content of biopsy specimens of dystrophic muscle for two hours after which time it slowly rises presumably because the muscle s thirst for creatine has been slaked Hottinger [190 claims that giving 12 to 18 mg of alpha tocopherol daily to healthy children immediately decreases their normal creatinuria and also that caused by glycine though that caused by creatine is not affected In deficient

rabbits hepatic creatine is increased Heinrich and Mattill [194] suggest that this is due to increased synthesis by the liver and failure of phosphorylation by the muscles Allantoin excretion is increased six fold in deficient monkeys [92]

Fat and protein metabolism are both discussed later on p 620, and blood clotting on p 622

Tissues from vitamin E deficient animals show a multitude of changes In calf muscle [131] potassium is decreased and sodium increased presumably because of the oedema (p 619) There is a true decrease in the total nitrogen with an increase in stroma protein and a decrease in muscle albumin There is only an apparent increase in fat since while the total and free cholesterol and the lipoid phosphorus fraction are increased the proportion of these to the total fat remains normal Roughly similar observations have been made on rat and guinea pig muscle [177, 192] and further glutamine is grossly decreased in rats and slightly in rabbits [186], but all these investigations are not truly helpful in understanding the true nature of the metabolic disaster since by the time a muscle has become dystrophic its analysis only reveals the sum of the compounds which occur in an uneven mixture of normal degenerating necrotic and calcified muscle fibres and wandering cells and connective tissue The same might be said about the fall of glycogen which is stated on rather slight evidence to occur in the muscles heart and liver [193] though it is of interest to note that vitamin E may be necessary for the formation of the glycogenic hormone of the pituitary (p 611) Butturini [407] states that vitamin E in animals and diabetics increases the difference between the levels of sugar in arterial and venous blood causing storage of glycogen in the muscles liver and heart Vitamin E has been reported to raise the blood sugar curves of normal children [190] The very important part vitamin E may play in warding off senility is discussed on p 622

Isolated Tissues The oxygen consumption is increased in dystrophic muscles [117 186 187 189] and in muscles from the deprived hamster before the onset of dystrophy [186] adding glucose or diphosphopyridine nucleotide to the nutrient medium makes no difference [186] while some workers claim [117] and others deny [197] that the addition of alpha tocopheryl phosphate reduces the oxygen consumption Homogenates of muscles from deprived rabbits and guinea pigs show decreased activity of aspartic glutamic transaminase which is not increased by the addition of pyridoxal phosphate [198] However, no decrease in six enzymes in the muscle and two in the liver from dystrophic rabbits was found by Jacobi and others [203]

Isolated Enzyme Systems Minute amounts of alpha tocopherol stimulate acetylcholine synthesis [199] but generally tocopheryl phosphate is used for investigating enzyme systems because it is water soluble and so can be added to aqueous preparations However Ames and Risley [200] have pointed out that the *in vitro* effects of alpha tocopheryl phosphate probably bear no relationship to the biological functions of vitamin E This is partly because the phosphate inhibits practically every enzyme on which it has been tested partly because alpha gamma and delta tocopheryl phosphates have the same inhibiting effect on succinoxidase systems [20 [illegible] ecause the phosphate combines with prot [illegible] combines non specifically with an act [illegible] Similar conclusions have been reac [illegible] king with succinoxidase systems [202] though Jacob [illegible] others [203] in 1950 claimed to have shown that part at least of the inhibition of this enzyme is a specific effect They also report no inhibition of several other enzymes References to and brief reviews of the various enzyme systems which have been investigated up to about the end of 1949 have been given by Ames and Risley [200] and Ames and Harris [201] When an alpha tocopherol protein conjugate is

added to an enzyme system it probably can exercise its normal biological function in the case of a succinoxidase system it gives protection probably merely acting in its classical role of an antioxidant [200] In an important paper Miller and Dessert [204] suggest that vitamin E may regulate hyaluronidase activity and that the immunity of the immature testis to deprivation of vitamin E is due to it not yet having hyaluronidase while the mature testis suffers because in the absence of vitamin E the activity of its hyaluronidase is unchecked One man had no hyaluronidase in his semen as long as he was given vitamin E

ESTIMATION OF VITAMIN E

The cumbersome and costly biological method of estimating vitamin E by its effect in preventing resorption gestation in rats has been largely supplanted by highly complex but more concise physico chemical methods though it must remain the basic yardstick for checking the accuracy of all types of estimation

Biological Estimation The foundations of the biological method were laid by Evans and Burr [2] the criterion of vitamin E activity used being

FIG 210 The uterus on the left is from a pregnant rat on a normal diet while that on the right is from a pregnant rat on a diet deficient in vitamin E Note the smaller number of embryos within the latter and their arrested development

the cure or prevention of the particular kind of sterility brought about by deprivation of vitamin E (p 606) These authors pointed out that the only certain method of knowing that a doe on a vitamin E deficient diet was really depleted of vitamin E was for her to have a typical resorption pregnancy Only then was such a doe fit to be given the dietetic supplements whose vitamin E content was being examined It was necessary to mate her with a buck of proved fertility and to make certain that both positive mating and

a litter was born it was good proof that
tamin E Time could not be saved by
gestation because the animals varied

so much in their initial stores of vitamin E that even in litter mates kept on

the same deficient diet, some rats might show "first litter fertility," and some might not.

Bacharach [23], however, has perfected a technique by which the preliminary absorption gestation can be omitted, with great saving of time. He stresses that there are "very large and unpredictable variations in the average response of groups of animals at different periods even though the pre-experimental and experimental conditions have, as far as possible, been kept constant." He found, for instance, during one series of experiments that the mean fertility dose of a substance appeared to increase threefold [7]. This emphasizes the importance of using racemic alpha tocopheryl acetate as a control during all estimations. Bacharach [24] has also worked out a dosage response curve for vitamin E which shows that in comparing two substances for their vitamin E activity accurate comparisons are only possible when the fertility rate of the animals is about fifty per cent.

Originally it was necessary to adopt the "all or none" law for fertility, rats either did or did not produce a litter. This meant that a graded response to vitamin E supplements in the diet was not provided. But Mason [7] overcomes this difficulty by killing all assay animals on the sixteenth day of pregnancy and examining the number of living and dead fœtuses and resorption sites. From this he works out the "uterine index," which shows a graded response to graded supplements of vitamin E [25]. A somewhat similar technique is used by Homrich [26], who emphasizes the importance of a fat free diet, of giving the test substance after mating instead of before, of discarding animals which have less than seven or more than fourteen implantation sites and of not using bucks with a poor breeding record.

Gottlieb, Quackenbush and Steenbock [27] have found in rats that, with a diet low in fat, the increase in weight during pregnancy is, within limits, in direct proportion to the amount of vitamin E supplied. This method of assay is economical, since the animals can be used again and few are needed because of the graded nature of the response. It is also claimed to be more sensitive than other methods, enabling amounts of vitamin E to be determined which are too small for the production of litters.

Other biological actions of vitamin E have been suggested for use in its estimation: thus Herraiz and Radice [28] have done preliminary work with the testicular degeneration which occurs in deficient young rats (p. 610); Mackenzie and McCollum [29] have drawn attention to the rapid decrease in urinary creatine of rabbits, rendered dystrophic by lack of vitamin E, when the vitamin is added to their diets (p. 615); Dam and Glavind [30] believe that the alimentary exudative diathesis which occurs in deficient chicks (p. 619) would be suitable; Rose and Gyorgy [31] have shown that the red blood cells of deficient rats are far more sensitive than those of normal animals to hæmolysis with dialuric acid—though according to Cater [32] this hæmolysis is much less marked with the red blood cells from deficient chicks, possibly because they are nucleated. Using hæmolysis, when it has been further investigated, promises to be of great value not only for easy and rapid assay work, without killing the animals, but also for indicating both in animals and man a deficiency which gives no overt signs.

Physico-chemical Estimations. Physical and chemical methods are generally combined when estimating the tocopherols, the former including molecular distillation, chromatography, photometry, spectroscopy, etc., while the chemical methods are most often based on the reactions first described by Emmerie and Engel [33] or Karrer and Keller [34] or Furter and Meyer [35]. In the first of these ferric chloride is reduced by the tocopherols, the reduction being measured by the photometric estimation of the red complex formed with α, α′ dipyridyl, while in the second method the reduction of the gold chloride is measured electrometrically. The third method is based on the red colour given by tocopherols with nitric acid.

Total tocopherols in oils and animal tissues, in blood and in foods may be

assayed by the methods described by Cuthbertson and others [36], by Tošic and Moore [37] and by Quaife, Dju and Harris [38, 39], whose papers should be read for the full and complicated details of the various techniques employed The first of these, and by far the least sensitive, is the only one based entirely on spectroscopic methods, the others being in essence chemical

Different foods contain different proportions of the four tocopherols, and as these vary greatly in all aspects of their biological activity (p 594) it is necessary to measure them separately if the value of foods is to be assessed with any accuracy. The problem is simplified by beta-tocopherol only

may introduce a slight but definite error when assaying the biological potency of a food To measure gamma- and delta tocopherols separately in a food also containing alpha tocopherol, o-dianisidine is employed which gives no colour with the latter and different colour intensities with the two former in different alkaline solutions [41] All four tocopherols may be measured in a food by the method described by Quaife [42], which in essence depends on estimating the total tocopherols and then making the nitroso derivatives of beta , gamma- and delta tocopherols,
and measuring each photometrically
qualitative and possibly for quantitati

PHYSIOLOGY OF VITAMIN E

Nothing is known of how plants synthesize vitamin E, though it is possible its origin is closely related to that of vitamin K [16] All animals so far investigated appear to be dependent on plant tissues for the vitamin, not forming it themselves Most probably the bacteria of the gut—at least of the rat [44] and cow [45]—do not synthesize vitamin E For insects the vitamin is important or perhaps essential [46, 47]

Absorption. Whether vitamin E, like carotene, undergoes any change within the wall of the intestine during absorption is not clear, though the matter may be important since Milhorat and Bartels [48] have suggested that the fundamental metabolic error in human muscular dystrophy is failure to conv
among
that vit
and cure of both sterility and muscular dystrophy. In the latter condition, however, Mattil [50] and others [51, 52] found alpha tocopherol more active when given by mouth than by injection There is no rise in the blood level unless it is dissolved in a " Tween " before injection, which may be dangerous for man [311] Wechsler and his collaborators [53] report that the level of vitamin E in the blood of three patients actually fell after intramuscular injections of 100 mg of alpha-tocopherol, though the level rose when the vitamin was given by mouth

There is strangely little information about how complete is the intestinal absorption of vitamin E Some escapes absorption and appears in the fæces of rats when the die
may be a quarter of
amount excreted by
between 1 and 100 mg , age making no difference, nor whether the tocopherol is in the natural or the racemic form [44] On the other hand, absorption is said to be more efficient in deficient animals [44] Hens excrete about three quarters of a single dose of 1,000 mg [62] Man also excretes part of the tocopherol he consumes [44] which on normal diets is said to be about two thirds of the intake [414]

Fat and the normal absorption of fat may be important for the absorption of vitamin E, a possibility which should be investigated now that fat-free forms are almost wholly used in medicine. But no direct work has been done on this, so that evidence must come from conditions where fat is poorly absorbed. Thus in sprue [55, 65, 71] and the steatorrhœas [71] the level of vitamin E in the blood is low, and five men have been reported [56, 61] who, after dying from "sprue" or chronic and profound digestive disorders, were found at post-mortem to have the tissue pigmentation, muscular dystrophy and testicular atrophy typical of experimental vitamin E deficiency. Two cases of steatorrhœa [57, 58] are said to have shown a remarkable clinical improvement when given vitamin E. Therefore, even if fat is not itself an important aid to the absorption of vitamin E, yet any conditions which hinder fat absorption are liable to affect vitamin E in the same way. But it must be remembered that rancid fat destroys vitamin E during digestion (p. 23).

Bile appears to be necessary for the absorption of vitamin E. Brinkhouse and Warner [59] report that dogs with chronic biliary fistulæ develop within seven to nine months both the nutritional muscular dystrophy and testicular degeneration typical of a deficiency of vitamin E. In one dog the muscular dystrophy was arrested and improved when bile was fed by mouth. Greaves and Schmidt [60] have obtained similar results in rats. The effect of hepatic disease in man on the level of vitamin E in the blood is discussed on p. 602.

Liquid paraffin probably hinders the absorption of vitamin E as it does that of vitamins A, D and K.

The placenta appears to offer no barrier to the passage of vitamin E, since the level of vitamin E in the plasma of women and in the *venous* cord blood plasma of their infants is virtually the same [63, 64]. As the level in the arterial cord blood plasma is only a half or a third of that in the venous cord blood plasma [63, 64], it would appear that the new-born infant destroys or stores vitamin E with amazing rapidity; so estimations of the level of vitamin E in "cord" blood plasma, without any care being taken to collect only arterial blood, are valueless. Not realizing this has led several investigators to the belief that vitamin E passes the placental barrier with difficulty, merely because "cord" blood plasma has been found to contain less vitamin E than the maternal plasma. Fœtal plasma levels and fœtal storage are discussed below.

The hen [62] transfers vitamin E to the egg in considerable amounts: on a commercial laying mash the vitamin E content may be as high as 4 mg per 100 grams of egg, of which about eighty-five per cent. is alpha tocopherol. The average content, however, is about 1.2 mg per 100 grams, which drops to somewhere between 0.16 and 0.64 mg before a vitamin E deficient diet stops laying, at which time the plasma level has fallen to about 0.1 mg. At intakes of 100 to 200 mg weekly the efficiency of transfer to the egg is for alpha-, gamma- and delta-tocopherols 16.5, 2.9 and 1.35 per cent.

Plasma Levels. The level of vitamin E in the blood is virtually always estimated and given as the level in the plasma, even when it is called the "level in the blood," though Bratzler and others [93], working with pigs, have shown that when the plasma contains no vitamin E the whole blood may yet contain about 0.087 mg per 100 ml, and when the plasma contains 0.67 mg the whole blood may contain 0.73 mg. This difference between plasma and whole blood might just conceivably be important, especially when considering levels in anæmic patients or those with polycythæmia, such as new-born infants.

The average level of vitamin E in the plasma of healthy human adults is about 1 mg per 100 ml, but there is a wide variation. In England [415] the mean value for 64 healthy subjects was 1.31 mg with a range of 0.60 to 2.91 mg and for 175 mental patients 1.15 mg with a range of 0.56 to 3.25 mg. Engle [65], from an investigation of 122 normal Dutch adults, found a

range of 0 3 to 1 3 mg while Harris and others [66] in the U S A consider 0 88 to 2 mg normal and below 0 88 mg subnormal basing this opinion on the blood levels of seventy normal adults and 350 who had a background of malnutrition and vague symptoms without any abnormal physical signs Similar levels have been reported by many other workers in North America [68 69 70] Sex [68 69] and negroid colour [68] make no difference though there is a tendency for higher levels in old age [68 71] especially in men [69] During pregnancy levels tend to rise in one extensive investigation [71] the average values for successive trimesters being 0 84 1 07 and 1 24 mg per 100 ml falling to 0 96 mg six weeks after delivery Other workers [70 75] give very similar figures for pregnancy and the puerperium with an average blood level at term of 1 7 to 1 8 mg There is no change during the menstrual cycle [71] Venous cord blood has roughly the same value as the mother s and arterial cord blood considerably less [63 64] while the new born infant has low levels of about 0 26 mg [72 73] though there are very wide variations Prematurity and weight at birth make no difference [72] but after five days the average level for full term infants has risen to an average of 0 36 mg while in premature infants no rise occurs for two months after which levels reach about 0 5 mg at three to six months [72] In nine children aged five to fifteen years the level was 0 72 to 1 12 mg [85]

Poor nutrition such as that which generally results from hospital diets [68] and poverty [66] tends to show itself in blood levels of 0 9 mg or less while during the German occupation and starvation of France [75] in 1943 the average values were 0 2 mg The German starvation of Holland had the same effect [76]

Daily oral doses of synthetic racemic alpha tocopheryl acetate may raise the plasma level very considerably or may have little effect presumably depending on individual differences in absorption storage and destruction Thus 600 mg daily for one month raised the levels of twenty one cases from an average of 1 2 mg to an average of 2 95 mg —values ranging from 1 64 to 4 69 mg —which fell after a month to 1 66 mg [67] With doses of only 150 mg daily the average level reached was 1 76 mg with a range of 1 28 to 2 73 mg High values and wide individual differences after dosing have also been found in premature infants who with an average level of 0 25 mg may show no increase with daily doses of 150 mg or may have levels above 4 mg [73]

Tolerance curves after single oral doses again show marked variations thus Klatskin and Krehl [77] using 500 mg doses found in twenty one subjects that the maximum rise occurred any time between three and thirty six hours after the dose the increase in some cases being only 0 04 mg while in others it was 3 63 mg The average rise was 1 31 mg and this occurred most frequently between six and nine hours after the dose Levels were still slightly elevated in many cases after seventy two hours

The cerebrospinal fluid contains no vitamin E [76 78]

Illness has strangely little effect on the level of vitamin E in the blood in spite of the spate of claims that many diseases are benefited by vitamin E (pp 632 661) Indeed the only two groups of diseases which appear to have any effect on the level are firstly those associated with chronic steatorrhœa (p 600) where the level is low due presumably to impaired absorption and secondly those associated with hypercholesteræmia where the level is often high [71] which may be the reason why the disease of old age tends to have high levels [68 71] especially in men [69] But there is no absolute relationship since in hepatitis the cholesteræmia and vitamin E levels do not always fluctuate together [77] Whether a high level of vitamin E causes a high level of cholesterol or *vice versa* is obscure in favour of the latter are observations on diabetics in whom a high level of cholesterol appears to be always accompanied by a high level of vitamin E [82] while raising the level of vitamin E does not raise the level of cholesterol [83] , nor

does it in rats [288], though it does so in rabbits [84] and is said to do so in schizophrenics [375].

Of the conditions for which vitamin E has been most frequently advocated, the muscular dystrophies and neurological degenerations tend to have normal [65, 85] or low levels [53]; cardiovascular diseases, normal [68] or high [67, 71, 79] levels; diabetics [82, 83], cases of primary fibrositis [153] and infants with retrolental fibroplasia [73], normal levels, while in the complications of pregnancy Scrimshaw and his colleagues [80] compared the blood levels of 197 normal women at various stages of pregnancy with the blood levels at the same stages of pregnancy of sixty-nine pre-eclamptic women, another twenty-five who had premature infants and a further sixteen whose pregnancy was complicated by essential hypertension. They found that none of these women had abnormal levels. In contrast Rauramo [81] reported that, of thirty-two women with levels below 0·6 mg. per 100 ml., forty-four per cent. developed toxæmia, nineteen per cent. had hyperemesis and forty-one per cent. gave a history of previous abortions or premature delivery; on the other hand, women with levels above 0·8 mg. had respective percentages of 8, 14 and 14. The part played by vitamin E in human pregnancy is further discussed on p. 633.

Hepatic disease gives a flat tolerance curve [77, 79], but in the very thorough investigation carried out by Klatskin and Crehl [77] "the plasma tocopherol level could not be correlated with the degree of hepatic dysfunction, the type of liver damage, the presence of jaundice, the serum cholesterol concentration or age." Levels are high in nephritis [65, 71, 79], normal in nephrosis [79] and are said to be low in Leber's optic atrophy and hyperplastic uterus [65]. A few cases of most medical conditions have been investigated [71, 77, 79] and levels appear to be within normal limits in virtually every disease not already discussed, including pneumonia [77] and infections [71].

Animals vary greatly in their levels, the following average plasma val being milligrams per 100 ml.: *macaca rhesus* monkeys [86] 0·58; hor and mares [88] 0 to 0·4 and 0·15 to 0·34, whether stall fed or at pasture, wi very slightly lower levels during pregnancy and normal levels in sterility; cows [88] 0·1 to 0·2 when stall fed and 0·8 when at pasture, the levels fallin slightly with age but not altering with pregnancy, parturition, *Brucell* infections with or without abortion, sterility, nymphomania and anaphrodisia, though other workers [89, 102] state—probably correctly—that there is a fal shortly before pregnancy such as occurs in women (p. 601); new born calves [89] 0·04; lambs [90] 0·02; kids [90] 0·016; farrows [90] 0·12; pigs [93] plasma 0·63, and whole blood 0·69; sheep [94] 0·33; rats [31] on stock ration 0·38, and on deficient ration 0·27; chickens [91] at 1·0 have complete protection against cod liver oil, and at 0·1 cease laying [62]. Supplementing the maternal diets raises the levels in new born calves, kids and lambs but not farrows [89], which suggests that, as the last animals are the only ones whose diets are very rich in vitamin E, all the others were really receiving an insufficient diet.

Storage. In the tissues of a man of thirty and a woman of forty-three, killed in an accident, the following values respectively in milligrams per 100 grams were found [39]: muscle, 0·62 to 1·32 and 1·56 to 3·8; liver, 2·49 and 2·19; fat, 24·7 to 29·2 and 39·2 to 49·5; heart, 1·11 and 1·28; kidney, 0·8 and 3·32; pancreas, 5·49 and 10·6; spleen, 1·88 and 4·7; testis, 2·83; uterus, 1·47. The fat of the testis and the uterus, with 1,210 and 1,160 mg. per 100 grams, was the richest in the body, fat from adipose tissue containing only 297 to 359 mg. and 605 to 626 mg. An odd finding was that in the man gamma and delta tocopherols were only present in the subcutaneous fat, while in the woman they formed twenty to forty per cent. of the tocopherols in most tissues. Very similar values for muscle have been found by other workers, the highest level of 4·8 mg. being found in a case of myasthenia gravis [95]. In the livers of two patients who died from heart disease and

carcinoma of the colon the values were 3 37 and 2 64 mg while values of only 0 44 and 0 94 mg were found after death from cirrhosis and obstructive jaundice [79] The average content of the placenta of ten women was 0 41 mg which for eight women rose to 1 04 mg after dosing for three to thirty days with 90 mg daily of synthetic alpha tocopherol [64] Other workers report values of 0 56 to 1 07 mg on normal diets [96]

Animals can store vitamin E for a considerable period Evans and Burr [2] finding that a single dose of vitamin E might suffice the rat for three or even four normal pregnancies Biological assays by Mason [98] on the relative amounts of vitamin E in various tissues of the adult rat have shown that the liver stores large amounts when the intake of natural mixed tocopherols is high and less than any other tissue when the intake is low storage is never as great as that of vitamins A and D Active mammary tissue has the highest content of all organs and the heart lungs and spleen are twice as rich in the vitamin as the other viscera muscles and body fat

Chemical assays by Hines and Mattill [54] which they suggest fail to estimate all the vitamin in the tissues do not agree with Mason's findings since in rats whatever their intake the liver always contained large amounts of vitamin E compared to the muscles The values—expressed as milligrams of tocopherol per kilo of tissue—in animals receiving (1) a vitamin E concentrate equivalent to 100 mg of tocopherol daily (2) a normal diet which probably contained very little vitamin E and (3) a vitamin E deficient diet were for liver and for muscle 42 3 and 11 9 22 1 and 7 5 22 6 and 4 8 For rabbits the values were 86 8 and 28 1 9 2 and 8 9 4 and 5 7 Further figures for rabbits [97] are liver 9 to 14 muscle 1 3 to 3 3 brain 5 to 18 lungs 10 to 19 kidney 5 to 7 intestine 4 to 7 stomach 6 to 12 skin 2 6 spleen 10 and fat 6 Hines and Mattill [54] have suggested that the high and very slowly depleted hepatic stores of the rat may explain why this animal is more resistant to lack of the vitamin than the rabbit whose hepatic stores are low especially compared to those in the muscles

Lundberg and others [99] basing their assays on the effect of alpha tocopherol on the induction period [195] of abdominal fat found that storage in the fat of rats did not reach a maximum until seven or ten days after a single dose of 50 mg after which the amount stored fell to half its maximum in two months With an intake of 50 mg daily for ten days the maximum amount stored was 97 mg per kilo Alpha and beta tocopherols [100] were deposited in equal amounts the latter only reaching its maximum in fifteen days Much less gamma than alpha tocopherol was deposited in the abdominal and ham fats but not in the skin fat which is reminiscent of the deposition in male human skin mentioned above From these results it is suggested that the biological activity of the different tocopherols is not related to their antioxygenic activity but to the amount of each deposited in the tissues

The pig [93] from studies on only five animals has the following stores respectively of total tocopherols in milligrams per 100 grams when fed a diet deficient in vitamin E and the same diet supplemented with 55 mg per kilo daily liver 0 16 and 42 3 muscle 0 37 and 4 16 spleen 0 46 and 5 34 kidneys 0 25 and 2 79 heart 0 1 and 5 6 while the content of the various fat depots varied from 1 36 to 35 3 for ruffle fat to 0 and 17 6 for ham facing fat But all the above figures varied very greatly from animal to animal Figures reported for the ox [97] are posterior pituitary 0 9 to 1 1 anterior pituitary 2 6 to 3 brain white matter 2 3 and grey matter 1 2

The foetus has small stores of vitamin E since the young born of rats and rabbits on a deficient diet develop muscular dystrophy only slightly sooner than do the young born of normal animals when both are suckled by a vitamin E deficient doe [3 101] The tissues from newly born rats of mothers

on a normal diet contain only small amounts of vitamin E [2] while livers of newborn lambs contain 2 5 mg per 100 grams kids 1 04 mg and farrows 2 47 mg [90] Supplementing the maternal diets of the last three animals did not appreciably increase fœtal hepatic stores The newborn animal appears therefore from the figures given below to depend for its vitamin E on colostrum (p 629) and not its own stores

Excretion of vitamin E in the fæces has been discussed on p 599 while its excretion in the urine of rats only occurs when it has been consumed in very large amounts [36] and it has not been found in human urine [76] In human bile it is probably present in the same amounts as in the plasma [79] The amounts of vitamin E in colostrum and in milk are given in the food tables and the subject is also discussed on p 609 so that here it is only necessary to emphasize the high levels of vitamin E in colostrum

Destruction The depletion of stores of vitamin E was shown by Evans and Burr [2] to continue at the same rate whether rats became pregnant or not though vitamin E is excreted in milk (p 629) It thus appears that vitamin E is used in the normal processes of the body pregnancy not appreciably increasing the consumption When large amounts of vitamin E are taken they are rapidly destroyed where this occurs is obscure [36] though it may be in the muscles partly because vitamin E plays an important part in muscular metabolism (p 612) and partly because Pappenheimer and others (p 618) have shown that the vitamin is only essential for the integrity of working muscle and therefore is possibly metabolized and destroyed during muscular work

It is improbable that the first stage in the destruction of vitamin E is its oxidation to tocopherylhydroquinone (p 593) though this has been suggested because the blood of dogs contains 0 46 mg per 100 ml and that of man 0 31 mg per 100 ml [152] Hines and Mattill [54] however found none in the liver muscles and urine of rats consuming 100 mg of vitamin E daily which is a strong argument against vitamin E being oxidized to this substance

Requirements The need for vitamin E varies greatly with the composition of the diet highly unsaturated fatty acids for example increase requirements (p 615) while protein has the opposite effect (p 620) Therefore requirements are never a fixed amount but must always be considered against the whole dietetic background Hyperthyroidism [154] and old age (p 607) both increase the needs for vitamin E Further Harris [107] has suggested that requirements depend on physiological weight which for many activities of the body [108] is only seventy per cent of the physical weight This relationship can very roughly be used to deduce the needs of one animal from the known needs of another thus per kilo of body weight the lamb should and in practice does require seventy per cent of the vitamin E required by the mouse [107]

In the rabbit in which muscular dystrophy is the dominant effect of a deficiency (p 614) requirements depend on body weight—but not on age or sex [52]—being according to Mackenzie and McCollum [29] from 0 7 to 1 mg daily of alpha tocopherol per kilo of body weight Much smaller amounts allow of growth over a long period and ward off muscular dystrophy so that such mild dystrophic lesions as do occur give no clinical symptoms [103] Eppstein and Morgulis [52] state that doses as low as 0 32 mg daily per kilo of body weight are sufficient for all needs

In the rat in which loss of fertility is the dominant symptom of a deficiency (p 606) the male requires more than the female Evans and Emerson [104] gave rats of both sexes from weaning 0 1 0 25 and 0 75 mg of alpha tocopheryl acetate daily The smallest dose protected both sexes from muscular dystrophy and enabled the females to have three litters though ultimately there was uterine discoloration (p 607) and the suckling young all developed muscular dystrophy Daily doses of 0 25 mg had a

slightly better effect but even doses of 0 75 mg did not completely prevent dystrophy in the young of the third pregnancy In the males doses of 0 75 mg preserved fertility for at least sixteen months while doses of 0 25 and 0 1 mg preserved it for nine and five months Post pubertal testicular degeneration is considerably delayed but not prevented by a single dose of 0 5 to 5 mg when this is given on the fifteenth day of life when given two weeks later or one week earlier there is little or no effect [105] Similar protection is provided by giving the nursing mother 51 mg [106] Gottlieb and others [27] give the total requirements for a normal pregnancy with normal offspring as 0 85 to 1 5 mg when the diet contains no fat and double this when the diet is rich in fat while Rose and Gyorgy [31] judging by the resistance of red cells to hæmolysis (p 598) state that the rat needs 3 mg daily per kilo of body weight

Guinea pigs require 3 mg daily [109] lambs 0 23 to 0 37 mg per kilo [110] while Harris [107] at the end of 1949 collected the references to the scant literature on the needs of mice dogs calves and goats

Ducklings [124] require 2 to 4 mg daily to prevent muscular dystrophy laying hens [62] a minimum of 1 2 mg daily for egg production which will not give an egg rich in vitamin E, while chicks (p 619) vary in their needs with the composition of their diets

THE FUNCTIONS OF VITAMIN E

There is a remarkable variation in the response of different species to a deficiency of vitamin E though fresh work is continually showing that what at first seemed a variation in kind is really only a variation in degree different tissues in different species may suffer first from a deficiency but the ame tissues probably all suffer in the end if death due to the involvement f some essential organ does not supervene In the rat [2] the earliest sign of a deficiency is sterility in the male and failure in the female to carry pregnancy to term If the deficiency is prolonged changes occur in many organs showing that vitamin E is not solely concerned with reproduction but with most activities of the body The endocrine system and kidneys may be affected (pp 610 623) and the central nervous system degenerates (p 617) There is also a primary degeneration or dystrophy of voluntary and involuntary muscle (p 612) and extensive deposition of ceroid pigment (p 621) The mouse appears to differ from the rat according to current work [111] only the female requires vitamin E for reproduction—though this has been questioned [136]—only the young develop muscular dystrophy the central nervous system and kidneys do not degenerate and less ceroid pigment is formed In the guinea pig dystrophy of voluntary muscles dominates the picture [4] though failure to carry pregnancy to term [113] and testicular degeneration [114] can occur in animals rendered dystrophic but not killed by diets only partially deficient in vitamin E

A similar muscular dystrophy with or without ceroid is the earliest and as yet the only recognized symptom of deprivation of vitamin E in the monkey [86 87, 92] and rabbit [29 115] both of which also suffer myocardial damage and in the hamster [117 118] and tree kangaroo [116] The rabbit [137] and hamster [136] also suffer in time from testicular damage The dog develops muscular dystrophy [119] and probably testicular degeneration [59] and there is some evidence that foxes [120] also require the vitamin Cats [121] are said not to require vitamin E but this may be due to the deprivation not having been long enough to produce symptoms [119] Steatitis a condition which attacks minks appears to be muscular dystrophy with ceroid pigment [129]

The duck [124] develops dystrophy of voluntary muscle while in the turkey [125] it is the smooth muscle of the gizzard which is affected The chick suffers from encephalomalacia or an œdematous condition of the body

(p 619) and possibly muscular dystrophy [126] The laying hen ultimately ceases to lay [62] The testes of cockerels [127] degenerate and the development of the embryo in the egg is arrested [127, 128]

All farm animals, so far as they have been investigated, require vitamin E, though there is little information about the goat [107, 177] and horse [88] the paralytic myoglobinuria of the latter being possibly muscular dystrophy [130] In calves Blaxter, Watts and Wood [131] have produced typical muscular dystrophy on a diet of dried skim milk, arachis oil, lard glucose, vitamins A and D and minerals The dystrophy is prevented by 50 mg daily of alpha tocopherol, unless 16 ml daily of cod liver oil is given instead of the arachis oil—an interesting observation, since the Ministry of Agriculture and Fisheries advises giving calves four times this amount of cod liver oil The cow, as Gullickson and others [45] have shown, may continue in perfect health for four generations on diets which cause resorption gestation in rats But in spite of normal reproduction without abortions, in spite of normal growth and lactation, in spite of all this, the animals may suddenly die, generally in the latter half of pregnancy or shortly after calving Post mortem examinations show no cause for death, though electrocardiograms during life suggest it is due to myocardial degeneration [132]

Cotchin [130] has given an excellent review of "stiff lamb disease," or muscular dystrophy of lambs, and has described his findings in a flock in Berkshire The condition may attack large numbers of lambs when the ewes have been fed a poor ration during a hard winter Besides typical lesions in the voluntary muscles the myocardium is often involved, but the central nervous system is spared The ewes themselves are not affected, though there is slight evidence that a very prolonged deficiency may attack their muscles At least 50 mg of alpha tocopherol are required to cause a slow recovery, death is generally due to pneumonia or to starvation the lambs being too weak to suck Sows [135] suffer from resorption gestations and farrows from muscular dystrophy, and probably there is testicular atrophy

Tadpoles require vitamin E for growth [133], but the hastened metamorphosis induced by thyroxine is abolished [184] Insects probably require vitamin E [46 47], though a report [122] that the vitamin is present in the queen bee's royal jelly has not been confirmed [123]

In man a deficiency of vitamin E possibly causes abortion and muscular dystrophy, but whether or no the nervous system is affected is still obscure (pp 632 635 647)

From all these different manifestations of a deficiency of vitamin E it can be seen that the name "antisterility vitamin" is due purely to the chance use of rats in the first investigations Had guinea pigs, for instance, been first used the name "antidystrophic vitamin" would have been as widely adopted

In the following pages the functions of vitamin E will be considered separately in relation to the various systems of the body and to various metabolic activities, but in order to avoid repetition some subjects which are relevant to several aspects of vitamin E are only discussed in detail once, therefore the reader should consult the index to find the whereabouts of any particular subject

The Relation of Vitamin E to Reproduction Rats are the animals which have been chiefly used in studying the part played by vitamin E in reproduction The results of a deficiency of vitamin E in the female and male are so different that they need to be discussed separately

Reproduction in the Female Evans and Burr [2] stated that "In (female) animals reared on E free diets the processes of œstrus, ovulation fertilization migration and implantation take place in normal fashion but the young are never born, resorption occurring instead" This sterility, unlike that of the male, could always be promptly cured by giving vitamin E Later work, however, has modified these statements Martin and Moore [188]

report that "after prolonged deprivation of the vitamin the œstrous cycle became abnormal and it was impossible to render the animals pregnant. This failure to induce pregnancy is only due to failure of implantation [139] which is not surprising since there is in the uterus after prolonged deprivation a degeneration of the smooth muscle and brown discoloration due to small yellow granules in the muscle cells [138]. Further the uteri of rats which had been pregnant were often large and misshapen and metritis, salpingitis and ovarian cysts were common. Hessler [140] also noted similar changes in the uterus which occasionally spread to the vagina and ureters. The reaction of the uterine muscle was normal both to drugs acting on it directly and to those acting through its nerve supply. Barrie [141] has recorded pigmentation apparently increased by pregnancy and also fibrosis of the uterine muscle and in some cases fibromyomata. The longer animals were deprived of vitamin E the larger the amount they required for a normal pregnancy. A resorption pregnancy also increased the requirements of vitamin E. Females deprived of the vitamin tended not to mate. Vitamin E did not reduce the discoloration of the uterus unless pregnancy occurred — presumably because the increased uterine circulation of pregnancy is necessary to remove the pigment. The pigment is further discussed on p. 621.

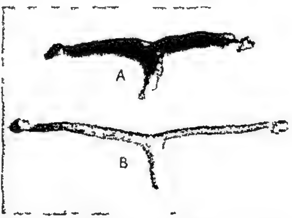


FIG. 211. Uterus A was taken from a virgin rat about fifteen months old which had received a diet deficient in vitamin E for about a year. Note the discoloration which contrasts with the normal colour of uterus B taken from a control of the same age and history which had received a supplement of two drops weekly of a preparation of the unsaponifiable matter of wheat germ oil.

FIG. 212. Uterus A was taken from a normal rat which had been used for breeding purposes while receiving an adequate mixed diet. Uterus B was taken from a rat which received a diet deficient in vitamin E and which had undergone pregnancy during routine vitamin E tests over a prolonged period. It is not only discoloured but permanently enlarged and misshapen.

Bacharach [142] has emphasized the increased requirement of vitamin E after a resorption pregnancy, and has also noted that in such animals the implantation rate is very low. The presumable explanation of the latter is the change brought about in the uterus itself from lack of vitamin E and fœtal resorption. The latter is the important factor since a moderately prolonged deficiency in virgin rats has no such affect.

Age itself also increases the need of vitamin E for reproduction about tenfold from the first half year of life to the second year [145] while the prolonged œstrous cycle of old rats is largely due to lack of vitamin E [146].

From all this it can be seen that lack of vitamin E exerts a profound influence on all the sexual mechanism in the female and not merely on the fœtal tissues as was originally held by Evans and Burr [2].

Lactation. Loosh [149] and Gullickson [45], among many others working with cows, have shown that vitamin E has no effect on the quantity or fat content of milk. Loosh has also briefly reviewed the relevant literature.

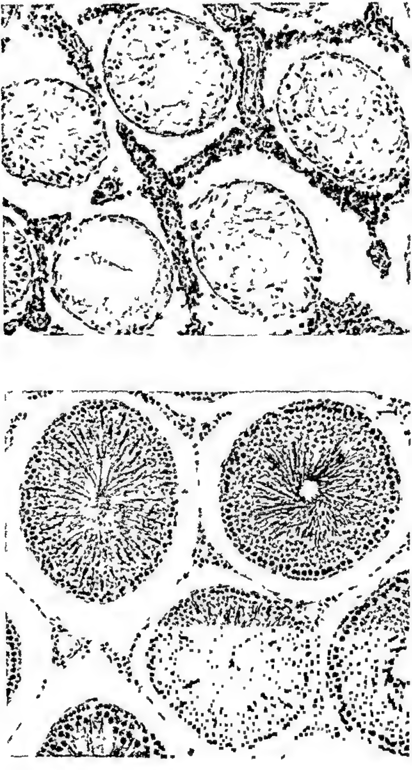


FIG. 213. Photomicrographs depicting the testes of litter mate *brothers* 277 days old fed on an identical vitamin E low diet, save that one *brother (photograph* 1) also received 0 75 mg of alpha tocopherol six times weekly. (For details see text, p. 610)

In rats, also, the balance of evidence is against vitamin E being necessary for milk production [141]. Of course the amount of vitamin E in milk (p 629) depends on how much is eaten.

Reproduction in the Male The following account is taken from the work of Mason [150] and Evans and Burr [2] In male rats born of mothers on normal diets, and then at weaning placed on deficient diets, sterility occurs when the rats are fifty to one hundred and fifty days old If, however, such rats are also suckled by mothers on a deficient diet they never become fertile (see also p 605)

In the first stage of the sterility the sperm appear completely normal both as regards their morphology and motility Nor can any changes be seen in the testes Even at this stage, according to Mason [150], the sterility cannot be cured nor complete degeneration of the testes averted Evans and Burr [2], however, cured about one quarter of their rats when testicular changes were already marked (Fig 213) and Mason [136] has reported similar cures in the hamster

As the degeneration of the testes progresses the sperm lose their motility and then begin to fuse together, or appear as fused cytoplasmic masses with fused sperm tails After this no more sperm are produced, and the germinal epithelium of the testes continues to degenerate This degeneration is first seen in the most mature cells, leading to fusion of the mature sperm Then the spermatids and secondary spermatocytes show nuclear changes with liquefaction and segregation of the chromatin material This is followed by the cells fusing together to form giant cells containing as many as forty nuclei These giant cells tend to slough into the lumen of the tubule Similar changes occur in the spermatogonia but the Sertoli tissue undergoes little change There is a marked difference in the rapidity of the degeneration in different tubules The testicular changes brought about by deprivation of vitamin E are typical and never seen in any other condition [150] Sexual interest is preserved for a long period after the onset of sterility, but is at length lost [2 150, 151] as it is in the female (p 607) Cater (p 625) states that in chicks the first effect of deprivation is precocious stimulation of the germinal epithelium, which is the cause of the ultimate atrophy

Other animals in which testicular degeneration has been reported are mentioned on p 605 and Mason [150] believes he has seen it in human testes as do others in sprue (p 600) Hyaluronidase (p 597) may play an important part in testicular degeneration

The Relation of Vitamin E to the Endocrine Glands Drummond, Noble and Wright [151] reviewed the work on the relationship between vitamin E and the endocrine glands in 1939 and reported the results of extensive investigations of their own They stated that "the experimental results which have been obtained do not indicate that the endocrine system is primarily responsible for the changes observed after feeding rats on an E deficient diet"

Testis The testicular degeneration described previously was again confirmed by the above authors, who found that its progress could not be arrested with vitamin E concentrates alone or in combination with injections of extracts from human pregnancy urine or pregnant mare serum The latter when injected into vitamin E deficient hypophysectomized rats also had no effect on the germinal epithelium, though the interstitial tissue responded That the interstitial tissue of vitamin deficient animals continued to function is shown by the weight of the prostate and seminal vesicles remaining relatively normal This is further shown by the normal reaction to drugs of the smooth muscle of the *vas deferens* and seminal vesicles from vitamin E deficient animals, which is quite different from the reaction of the smooth muscle from castrated animals [155] Sexual interest, however, is ultimately diminished [2, 150 151] Mason [150] agrees that the testicular damage is not a secondary effect from the pituitary, since the latter's removal does not cause the typical degeneration seen after deprivation of the vitamin Spermatogenesis in immature rats is not stimulated by vitamin E [160] Castrated rats survive longer than normal rats when both are deprived of

vitamin E [156] but this is probably merely an example of decreased metabolism decreasing the need for vitamin E even though testosterone has been reported to delay the onset of muscular dystrophy in vitamin E deficient rabbits [159]

Adamstone [157] found that vitamin E reinforces the action of testosterone in caponized male fowls this could well be due simply to the vitamin protecting testosterone from oxidation in the same way that it appears to [158]

ad alpha tocopherol when effect in rats Beerstecher [162] reports that the œstrogens in the urine of vitamin E deficient rats is normal during their pregnancy until resorption occurs when it drops sharply Drummond and others [151] observed no effect from giving vitamin E on the genital organs of young rats or those of adult hypophysectomized rats The uterus in spayed animals responds equally to injections of an œstrogen whether or no the animals are deprived of vitamin E [164] Ceroid pigment (p 621) does not develop in the uterus of the deficient spayed animal—as long as the diet is low in unsaturated fatty acids—unless an œstrogen is also given [165] presumably because the metabolic activity of the infantile uterus is so low The absorption gestation of vitamin E deficient rats cannot be prolonged to term by œstrogen [151 166] The effect of vitamin E on œstrus in old or deficient rats is discussed on p 607 and the effect of a deficiency on the ovary of the mouse on p 608 Minot and Dodd [163] have reported that the urines of seven boys with muscular dystrophy all contained œstrogens while the urines of normal boys did not

Progesterone The resorption gestation of vitamin E deficient rats cannot be prolonged to term by progesterone [151 166] which is not surprising as the *corpora lutea* only degenerate after the death of the fœtuses [166] In norm xcretion is not affected by 50 to onth [167] but when 60 mg are gi women before an injection of progesterone the subsequent excretion of pregnandiol is considerably larger than that following an injection without any previous tocopherol this is almost certainly due to the tocopherol preventing the oxidation of progesterone [158]

Thyroid hypoplasia has been reported by Barrie [52] and Singer [55] in rats and by Anderson and others [44] in dogs Singer [55] found the hypoplasia did not respond at all to iodine and so little to injections of pituitary extract that she did not think it a pure anterior pituitary effect Mason and Bryan [56] on the other hand could not find any changes in the thyroids of young deficient rats nor could Telford and his collaborators [57] in rats at any age There appears to be no explanation of these contradictory reports In chicks [154] the need for vitamin E is increased by thyroxine while in tadpoles [134] minute amounts of vitamin E abolishes the stimulating effect of thyroxine on metamorphosis

Pituitary The pituitary both in function and structure has been reported to be affected by lack of vitamin E Barrie [143] and Underhill [7] state that the anterior pituitaries of both young and old rats show degranulation of the acidophils a spongy appearance of some of the basophils and considerable numbers of empty cells which are presumably acidophils They think that a deficiency of vitamin E hinders the formation of gonadotropic thyrotropic and galactotropic hormones This would explain the condition of their young rats which had very soft poorly ossified skulls and cretinism with hypoplastic thyroids it also explains the difficulty adult rats may have in suckling Singer [168] however failed to prevent the thyroid hypoplasia with pituitary extracts and mammary regression occurs only after fœtal resorption [166] Most workers also stress the normal appearance of young rats even when these have muscular dystrophy

Nelson [161] and Mason [150] have reported castration changes in the pituitaries of vitamin E deficient male rats leading to an increase in the factor for stimulating the female genital system in young rats. Since no such changes were found in the pituitaries of female rats, those of the males were thought to be secondary to the testicular degeneration. Rowlands and Singer [170] however tested the activity of pituitary extracts from female deficient rats on rabbits in œstrus instead of young rats. From this they deduced that lack of vitamin E caused a decrease in the luteinizing or ovulation producing substance but not in the follicle stimulating substance. These results have been largely confirmed by Drummond, Noble and Wright [151] using hypophysectomized rats as the test animals. Pan and others [171], however, report that in deficient animals of *both* sexes gonadotrophin is increased when hypophysectomized young males are used as the test animals.

Kepinov [172] has observed that in frogs vitamin E is necessary for the formation of the glycogenic hormone of the anterior pituitary. In starved or hypophysectomized frogs injections of adrenaline cause no mobilization of glycogen from the liver, but after vitamin E is given adrenaline produces glycogenesis in the starved but not in the hypophysectomized frogs. Sugar metabolism is further discussed on p. 594.

The resorption gestation which always occurs in vitamin E deficient rats cannot be prolonged to term by giving injections of whole pituitary glands, by implanting two fresh pituitary glands every other day or by injections of extracts of pregnant mare serum or human pregnancy urine [151].

The weights of the pituitary, adrenals, ovaries and uterus of deficient animals remain normal, while those of the prostate and seminal vesicles are not greatly reduced compared to the diminution of the testes [151]. A longer list of organs is given by Copping and Korenchevsky [7] who also report that the thymus is larger in both male and female vitamin E deficient rats. Tonutti [172] has reported involution of the adrenal cortex.

From a consideration of the results obtained it appeared very unlikely that the effects of E deprivation could be explained by an alteration in function of the anterior pituitary gland, the slight changes recorded being in all probability related to a secondary effect on the pituitary [151].

Clinical work on vitamin E and the sex hormones is discussed on p. 639.

The Relation of Vitamin E to Growth. Impaired growth is one of the earliest symptoms of a deficiency of vitamin E both in the embryo (p. 608) and the young animal. Evans and Burr [2] noted that the growth of rats was affected by the amount of vitamin E in the diet, and Copping and Korenchevsky [7] state that "the final body weight, total gain in weight and amount of fat deposition were less in the rats deprived of vitamin E than in the corresponding controls." Barrie [143] has emphasized the poor growth of her young rats, and Nelson and others [174] have reported that on diets deficient in vitamin E rats stop growing, to begin again when alpha tocopherol is given. Emerson and Evans [175] bred four generations of rats on diets low in vitamin E. Each successive generation grew less than the last.

Rabbits may cease to grow days before any other symptom of a deficiency appears (p. 614). Foxes [120], dogs [119], insects [46, 47], tadpoles [133] and children (p. 647) also probably require vitamin E for proper growth. The relation of vitamin E to fat and to protein metabolism is discussed on p. 620.

The Relation of Vitamin E to Muscular Dystrophy. A degeneration or dystrophy of the voluntary muscles occurs in virtually all animals (p. 605) which have been deprived of vitamin E for a sufficiently long period, and also possibly in man (p. 635). This degeneration is a specific result of deprivation of vitamin E, never following deprivation of aneurine, riboflavine, pantothenic acid, pyridoxine, vitamin A or protein [255]. However, lack of any of the last three added to lack of vitamin E greatly intensifies the degeneration, though lack of the first three makes no difference [225].

... mary effect of lack of vitamin E ... about by changes in the nervous ... on p 617 ... discussed under the headings of (a) the clinical picture (b) the muscular degeneration, (c) cardiac failure (d)

Fig 214 Photomicrographs of muscle removed for biopsy from the calf of an English boy aged seventeen with pseudohypertrophic muscular dystrophy In the top fibre transverse striations are barely evident and the left hand portion shows well wrinkling and longitudinal fraying In the second fibre these changes are more marked transverse striations have completely vanished wrinkling and fraying are very extensive and there is considerable sarcolemma nuclei proliferation The other fibres show varying degrees of similar change

Fig ...

the changes in metabolism (e) rancid fats their destruction of vitamin E and their relation to muscular dystrophy (f) the cure of muscular dystrophy

The Clinical Picture Evans and Burr [3] first reported a mysterious paralysis which appeared in the suckling young of rats deprived of vitamin E Suddenly towards the end of weaning the young who till then had appeared normal became weak lost weight and sometimes died in a few hours A few animals escaped altogether and some spontaneously recovered without treatment Others again did not die but remained in good health

though partially paralysed. In adult rats muscular dystrophy (Fig. 216) develops very slowly [5, 6, 138] But the rat is not a satisfactory animal to use when investigating muscular dystrophy because a nervous degeneration may obscure the picture (p 617)

Guinea-pigs and rabbits, on the other hand, develop muscular dystrophy without any neurological complications (p. 617) The condition appears within a few weeks on a deficient diet and, unless vitamin E is given, always progresses rapidly to death Generally the onset of muscular dystrophy prevents deficient rabbits from reaching sexual maturity, but in a few dystrophic animals Pappenheimer [116] has reported the birth of living young, and here muscular dystrophy was present at birth It is interesting to note in passing that Mackenzie and McCollum [29] point out that experiments purporting to show rabbits do not need vitamin E for reproduction must be fallacious as the mothers would die of dystrophy before complete depletion of vitamin E could prove it unnecessary for reproduction

The Muscular Degeneration Goettsch and Pappenheimer [4] in 1931 first described what they termed "nutritional muscular dystrophy" in guinea pigs and rabbits when these animals were deprived of vitamin E They wrote " . in the more chronic cases . . . these muscles with replacement fibrosis and lipomatosis closely resemble those of progressive muscular dystrophy in man." In 1940 Pappenheimer [116] in an excellent review stated "The disease may run a chronic course. In such animals comparatively few fibres are destroyed at any one time, but their gradual loss and replacement by fat and fibrous tissue brings about a picture which is identical with that of an advanced case of human muscular dystrophy." *Olcott [178] has described the pathological changes in the muscles of young* rats (see Figs 214 and 215)

Not all muscle ... normal while others rapid ... fibres is loss of their transv... going on to complete degeneration Polymorphs and histiocytes appear, the latter often fusing into giant cells Many fibres make attempts at regeneration , in some the nuclei increase and the muscle sul... making fresh small fibres ; in others the nucl... sarcolemma, so that a tube of nuclei is formed start to reform new muscle fibres. These efforts at regeneration go on even *in the midst of the most intense degeneration* [4, 116, 138] The pigment which occurs in dystrophic muscles is discussed on p 621 Hoagland and others [179] have described the changes in human dystrophic muscles shown by ultra-violet photomicrography.

Cardiac Failure, in spite of the absence of pathological changes in the cardiac muscle of rats [178] or in their electrocardiograms [180], appears to be the reason for the sudden death of animals with muscular dystrophy , a death which it is hard to explain by the condition of the voluntary muscles In rabbits with muscular dystrophy foci of acute myocarditis may occur together with electrocardiographic changes [115], while Houchin and Smith [181] have shown, by well controlled work, that dystrophic rabbits die from doses of posterior pituitary extracts which are harmless to normal rabbits On the other hand dystrophic rabbits not only tolerate doses of digoxin and ouabain which are normally lethal, but also have their lives prolonged by such doses These findings appear to be only explicable on the assumption that the heart of the dystrophic rabbit is so severely damaged that the decrease in the cardiac circulation brought about by the pituitary extract precipitates cardiac failure, while the different toxicity of the cardiac glucosides in the normal and dystrophic animal is that which would be expected if they were acting upon a normal or upon a failing heart Houchin and Smith [181] suggest that lack of vitamin E may be a factor in the sudden cardiac failure of beriberi, and may also be of importance in the nutrition of the human

heart, a subject discussed on p. 657. In deficient cows (p. 606) sudden cardiac failure occurs, preceded by electrocardiographic changes, though there is little structural damage to the heart, while in dystrophic lambs (p. 606) there may be gross damage. In deficient monkeys there is virtually no damage [87] but there are changes in the electrocardiogram [86, 92]: the amplitude of the R and T waves is reduced with inversion of the latter [86], and also there is shortening of the time for the initiation of ventricular ejection [86] The effect of vitamin E on the vascular system and blood is discussed on p. 622.

Changes in Metabolism. The metabolic changes brought about by lack of vitamin E are discussed on p 594, so that here it is only necessary to draw attention to the way in which the creatine in the urine reflects the disturbed metabolism of the muscle Just as dystrophic changes may be found in the muscles while the animal yet appears normal [103, 214], so may the creatine rise in the urine before any clinical change can be seen. Ni [182] was the first to apply to research in muscular dystrophy the well known fact that urinary

investigated the subject and report that the best guide to the approach of muscular dystrophy is the rise in urinary creatine. This may occur while the increase in weight and the appetite are still satisfactory. The drop in

dramatic and precedes clinical

It occurs in from twenty four

ased destruction and increased

Rancid Fats their Destruction of Vitamin E and their Relation to Muscular Dystrophy Rancid fats rapidly destroy vitamin E by oxidation. This destruction is most liable to occur when vitamin E is in the form of the synthetic vitamin or concentrated preparations, since then it is no longer protected against oxidation by the anti-oxidants found associated with it in such natural sources as whole wheat germ [215]

It is important to realize that as vitamin E itself is an anti oxidant it will be destroyed by fats before they become rancid through auto oxidation In other words a fat need not be rancid, certainly not smell rancid, before all its vitamin E is destroyed Further, *rancid fats can destroy vitamin E during digestion as long as both are fed together or within a short time of one another* [50, 103, 216] Claims that rancid fat given by mouth or by injection destroys vitamin E already absorbed into the body [217, 218] require further confirmation, probably being incorrect [99, 219] If substantiated they are an argument against using ketogenic diets in any medical treatment

The destruction of vitamin E by fats, due to their auto-oxidation, was investigated by Cummings and Mattill [220], who state that "the oxidation of unsaturated fats by atmospheric oxygen causes the formation of substances which impart to those fats a characteristic acrid odour, usually described as rancid" Rancidity, however, is difficult to define some of its products (p 671) are free fatty acids, aldehydes, ketones, and peroxides Some substances having hydroxyl groups, such as wheat germ and other vegetable oils, *retard rancidity and so protect vitamin E, but ultimately* wheat germ oil itself becomes rancid The fats commonly used in food are auto oxidizable in the following order cod-liver oil, lard, butter. Margarine is compounded of sundry fats and hydrogenated oils far from destroying vitamin E it may even possibly be a good dietetic source, though it has many nutritional disadvantages

From the point of view of the diets used to produce muscular dystrophy

this destructive action of fats is most important Not realizing this has led to much confusion. When Goettsch and Pappenheimer [4] first reported nutritional muscular dystrophy, they did not consider a deficiency of vitamin E was the cause because wheat germ oil supplements failed to prevent the dystrophy. It can now be seen that this was because their diet contained so much cod-liver oil and lard that the vitamin taken in the wheat germ oil was destroyed during digestion [103]

Ni [222] also made a perplexing observation, when by adding Chinese donkey skin gelatine (ah chiao) to a vitamin E deficient diet he prevented and cured muscular dystrophy. This not only lent support to, but also gained support from, the treatment of human muscular dystrophy with gelatine (p. 646) The explanation, however, according to the further work of Ni [224], is that the ah chiao, being added to the diet as a powder, adsorbed pro oxidants and so prevented the destructive oxidation of vitamin E

The most serious result of not realizing the effects of rancid fat was that Morgulis and Spencer [221] were led to postulate that two factors were necessary to prevent or cure muscular dystrophy One was fat soluble and probably vitamin E, the other was water soluble being found in wheat germ and lettuce The evidence for this came from observations that wheat germ oil alone could not prevent dystrophy but wheat germ oil and lettuce or whole wheat germ could do so Much work has been devoted to investigating this "dual theory" of muscular dystrophy, which has now been proved wrong

The most thorough proof that deprivation of vitamin E is the sole cause of muscular dystrophy is given by Mackenzie and others [103] using the same diet as Goettsch and Pappenheimer Alpha-tocopherol given separately from the rancid food, so that no destruction of the vitamin could occur, prevented muscular dystrophy. Wheat germ from which vitamin E had been extracted (defatted wheat germ) had no effect Further, alpha tocopherol cured animals who had had no "water soluble factor," which proved that the cure was not due to stores of the latter in the body. To complete the destruction of the "dual theory" of muscular dystrophy it was shown that animals who received wheat germ oil mixed with the rancid ration became dystrophic, but if defatted wheat germ was added as well dystrophy did not occur the defatted wheat germ protected the vitamin E from oxidation This rounds off the proof that vitamin E is the only vitamin necessary to prevent muscular dystrophy and that the superiority of wheat germ over vitamin E alone, or as wheat germ oil, is solely due to its protecting vitamin E from destruction by rancid fats This, of course, does not mean that the other vitamins and protein which are synergistic with vitamin E from the point of view of muscular dystrophy and are also contained in wheat germ do not assist in preventing, though they cannot prevent, muscular dystrophy The animals discussed above were all on diets containing an abundance of all the vitamins except vitamin E

The Cure of Muscular Dystrophy Vitamin E cures and prevents muscular dystrophy in all the many animals mentioned on p 605, but it can only prevent and not cure dystrophy in adult rats (p 613) In the previous section it has been seen that the vitamin must be given so that it does not come in contact with rancid fat either in the food or in the stomach Where this may, however, occur the vitamin should be given as whole wheat germ, because in this form it is given some protection against oxidation Apart from this it is unimportant what preparation of vitamin E is used—whole wheat germ, wheat germ oil as long as it itself is not rancid, or alpha tocopherol The latter may also be given by injection, but is less potent than when given by mouth (p 599) The value of the other tocopherols and the esters is discussed on p 594 The importance both of protein and of vitamin E synergists which are of course present in wheat germ must not be forgotten

Vitamin E acts with amazing rapidity Within twenty four hours the creatine in the urine may drop and in a couple of days may be normal The appetite and weight of animals improve as the creatine falls and a normal rate of growth is re established in one or two weeks Even when the animals are so weak they cannot stand or feed themselves vitamin E leads to a rapid return of strength in one or two days and by the end of a week few symptoms of the dystrophy may be left The cure of such animals is permanent and complete All trace of even severe dystrophic changes may vanish from the muscles in a week [29 103 116] In children however recovery if it occurs may take years (p 641)

The Relation of Vitamin E to Nervous Degenerations In rabbits and guinea pigs as has been seen in the previous sections lack of vitamin E

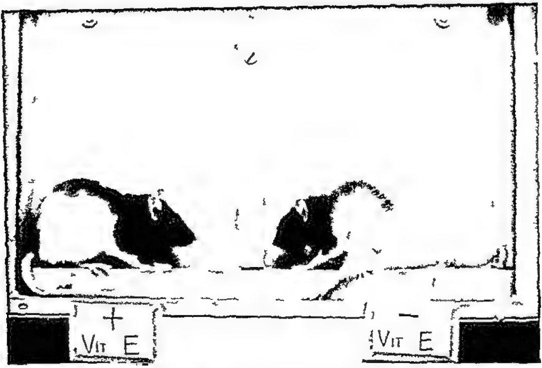


FIG 216 The rat in the right partition is a virgin female which received a diet deficient in vitamin E for about thirteen months It is emaciated rough haired and has paralysed hind legs The rat in the left partition is a control animal which received 3 drops weekly of the unsaponifiable matter of wheat germ oil

causes the rapid onset of a muscular degeneration which can be rapidly cured by the vitamin It is highly improbable that this degeneration is secondary to any nervous lesions because no changes have been found in the central nervous system [116] and the terminal neurites or end plates in the muscles remain normal even when the muscle fibres have completely degenerated [227] Further the rapidity of the recovery when vitamin E is given is far greater than could be accounted for by regeneration of nervous tissue In rabbits and guinea pigs therefore it seems most probable that lack of vitamin E causes a primary muscular dystrophy uncomplicated by any neurological degeneration though it must be admitted that some workers (p 618) have reported functional or anatomical changes in the nerves of these animals

In rats on the other hand lack of vitamin E probably affects both the muscular and the nervous systems so that a primary muscular dystrophy and a secondary muscular degeneration occur together The paralysis of

rats, however, is a complicated problem, partly because some observers
report extensive degeneration of the nervous system while others do not,
partly because young deficient rats may spontaneously recover without
vitamin E and partly because adult rats, though they develop paralysis very
slowly, cannot be cured by vitamin E nor even have the progress of their
paralysis checked This inevitable progress of the paralysis is more typical
of a neurological than a muscular disorder and is entirely unlike the primary
muscular dystrophy of the rabbit and guinea-pig, which can be rapidly cured

The outstanding work on the nervous system in deficient adult rats is
that by Ringsted [5] in 1935 and Einarson and Ringsted [6] in 1938 At the
date of their experiments pure synthetic alpha-tocopherol was not available
so wheat germ oil had to be given to their control animals Apart from this
probably unimportant point, their work and their histological technique
appear to be beyond criticism They reported that the initial lesion is a
degeneration, in the lumbar cord, of the proximal parts of the posterior roots
and the proprioceptive paths in the posterior tracts and probably in the
uncrossed tactile paths The amount of neuroglial reaction is
depending apparently on the rapidity of the ...
spinal ganglia are not aff...

[illegible]

co...stantly affected instead of only ... pyramidal tracts might be ... peripheral nerves degenerate and ... the death
of very recent ...
in the rat ...
on the who...
bination of ...
man, namely ... tabes dorsalis and ... the most important systemic ... state that resembles closely a com... We have,
If the rat had ...
and Ringste... ...occurring in
by giving ... ophy" [6]
review of th... Einarson
... whole subject was made by Einarson [416] in 1952 ... in its earliest stages ... nervous degeneration

Luttrell and Mason [228], Malamud, Nelson and Evans [229], Monnier [6] An outstanding
and Gutierez-Mahoney [231] have confirmed that lack of vitamin E causes a
widespread degeneration of the central nervous system of adult rats, though
their descriptions of the lesions differ and also do not exactly tally with those
given by Einarson and Ringsted ...
of various work... ...
in diet, a... ...ween the reports
siderably ... the final condition of the neurological degeneration, since differences
originally investigated and emphasized by Einarson and Ringsted [6] and ... will con-
has been confirmed by others [228, 229] ... of the nervous system. This was
similar changes in the nervous system of the young paralysed rat Lipschutz [233] has described

On the other hand, Olcott [178] Pappenheimer [116] and Wolf and
Pappenheimer [232] have all failed to find any chang...
of young or old paralysed rats ...
depri...
[6, 2...

T...
to be ... similar by Pappenheimer ... the muscles of paralysed rats have been found
Confirmation of the latter's work comes from the observations of Hines and
others [234], who showed that both in dystrophic rats and guinea pigs the ... and few in number by Telford [214]
tension in muscle developed after direct electrical stimulation was greater
than that after stimulation of the motor nerve De Castro and others [235]
claim that in rabbits changes in the central and peripheral synapses precede
the muscular degeneration

Cutting the motor nerve of a muscle protects it from the onset of

dystrophy [116 234] This effect cannot be due to any removal of an abnormal nervous stimulation since it also occurs when the tendon is cut [116] This strongly suggests that vitamin E is necessary for the active but not the resting muscle

Neuromuscular regeneration after crushing of a motor nerve is not delayed by a deficiency nor hastened by a super abundance of vitamin E [234]

Wheat germ oil is reported to give protection against the neurological symptoms of distemper and diphtheria

Davison s extremely important investigations (p 654) on human amyotrophic lateral sclerosis also strongly suggest that vitamin E is intimately connected with the central nervous system He treated ten cases with wheat germ oil and alpha tocopherol and in six of these post mortem histological studies (Figs 225 to 238) showed that in comparison with untreated cases there was less destruction of axis cylinders and myelin sheaths and considerably less gliosis There was no difference in the nerve cells of the bulbar nuclei and anterior horn cells

Vitamin E Deficiency in Chicks In chicks lack of vitamin E causes two quite separate conditions—nutritional encephalomalacia and the alimentary exudative diathesis

Nutritional Encephalomalacia This was first described by Pappenheimer and Goettsch [290] in chicks fed on diets deficient in vitamin E The chicks throve for about three weeks and then suddenly or slowly developed weakness ataxia tremors retraction of the head and other symptoms pointing to some nervous disorder Death was common but about two fifths of the chicks never developed the disease and others recovered without treatment in the same way that young rats with no treatment may recover from paralysis (p 613) As the birds grow older the condition becomes rarer never being seen in the adult [116]

All parts of the brain may be affected but the severest lesions are generally found in the cerebellum There is extensive ischæmic necrosis with œdema small hæmorrhages and hyaline thrombi in the small vessels in and about the necrotic areas The brain elements are disrupted with degeneration and necrosis of the cells Pappenheimer [116] states that while the arrest of the circulation is the cause of the condition it is impossible to tell whether the closure of the vessels was originally functional or caused by the hyaline thrombi The cholesterol content of the brain is said to be decreased [157] but this is probably incorrect [293] Adamstone [291] has compared the neurological degenerations caused by lack of vitamin A and vitamin E

Alimentary Exudative Diathesis This is the name given by Dam and Glavind [30] to a condition which appears between the sixth and thirtieth day in chicks reared on a diet low in vitamin E

Not all chicks are affected those that are die in six to eight weeks Protection is given by synthetic vitamin E There is a generalized œdema with fluid of the composition of blood plasma collecting in the subcutaneous and subfascial tissues especially those of the breast and abdomen The fluid also collects in the pericardial and peritoneal cavities The brain and lungs are œdematous and there is coronary and intestinal hyperæmia Deposits of urates are found in the kidneys and ureters This œdematous condition is reminiscent of that found by Pappenheimer [116 216] in young mice and rabbits born with muscular dystrophy

Dam [293] in 1944 reviewed his own work and that of others on these two deficiency diseases of chicks especially from the point of view of what factors decide which disease shall develop The amount and kind of fat in the diet is extremely important On fat free diets exudates seldom develop and encephalomalacia never Both conditions are accentuated by highly unsaturated fats fresh cod liver oil linseed oil and lard especially causing

exudates while the fatty acids from hog liver cause encephalomalacia Oleic acid and thoroughly rancid cod liver oil have no effect, but a mixture of *rancid and* fresh cod liver oil has the same effect as fresh cod liver oil This shows that it is the unsaturation of the fats and not their rancidity which is important An increase in the sodium chloride of the diet increases exudates and so does a certain carbohydrate protein ratio and also cholesterol though the latter gives protection against encephalomalacia Inositol [293] and

ons partial
nine nordi
Methylene
n the depot

fat and decrease peroxidation [-32] Ni [-24] reports that large amounts of vitamin A and carotene and possibly vitamin D increase the incidence of encephalomalacia presumably by increasing the intestinal destruction of vitamin E

The Relation of Vitamin E to Hepatic Necrosis and to Protein Metabolism Rats *deprived of both* vitamin E and sulphur containing amino acids develop acute massive hepatic *necrosis* [275 278] Casein however confers more protection against such *necrosis* than would be expected from its content of thio amino acids so that there would appear to be some additional protective factor in first class protein [275 277] *Confusion* in the past has been caused by some investigators *failing* to produce necrosis owing as Lindan and Himsworth [277] have *shown* to their not realizing that adult animals reared on stock diets or any animals previously given a high protein diet or large amounts of vitamin E are all protected for a long period against the effects of a dual deficiency of vitamin E and protein [277] Young rats especially males very frequently also develop massive lung *hæmorrhages* and distension of the subcutaneous blood vessels [275]

The reason for this hepatic *necrosis may* be the inability of the doubly deprived liver to detoxicate *poisons or toxins* Thus Gyorgy and others [278] have shown that *necrosis* is delayed from about forty days to about one hundred days by oral aureomycin terramycin and streptomycin The probable reason for this is that all these *drugs* destroy bacteria in the gut and so prevent the absorption of their toxic metabolites and their subsequent injury of the liver That these *antibiotics* do not directly aid the liver is proved firstly by *their* effect not being permanent owing presumably to the appearance of resistant strains of bacteria *in the gut* secondly by the effect of streptomycin which is not absorbed and which has its protective effect enhanced when its antibacterial effect is enhanced by mixing with pectin thirdly poorly absorbed sulphaguanidine also has some protective effect though the easily absorbed succinylsulphathiazole hastens the symptoms of vitamin E deficiency [279] Penicillin polymyxin and chloromycetin have no effect presumably because *they alter the flora of the bowel* in a different manner from the other antibiotics

Protein appears to be better *utilized* in the presence of vitamin E but in a very inconstant and *confusing* manner [272 273] for instance with a diet containing ten *per cent* of casein utilization is better but it is worse with a diet containing fifteen per cent of wheat gluten [273] Vitamin E is also stated to cause a hyperproteinæmia when fed to rabbits and rats *daily in* 30 mg and 10 mg doses [274] while lack of vitamin E has the opposite effect [280]

The Relation of Vitamin E to Fat Metabolism The destructive effect of the polyunsaturated fatty *acids* on vitamin F and the rôle which vitamin E *may play in preventing* the conversion of such acids into intra cellular *pigments* has been discussed on pp 615 and 621 while the influence of fat on the symptoms of vitamin F deficienc p 619
Vitamin E also plays a direct part *in th* Thus
mice [263] fed on a diet *rich in* fat but ne lean

while their liver cells contain abnormal, insoluble, pigmented "lipo proteic" globules, but no neutral fat On the other hand mice on the same diet with 2 5 mg of vitamin E daily become very fat and their liver cells contain excessive neutral fat, though very little of the "lipo proteic' substance Similar changes occur in the ovaries of mice [112] and Copping and Korenchevsky [7] long ago noted that deprivation of vitamin E reduced the amount of fat laid down by rats The changes in the muscles of dystrophic calves are discussed on p 596 Both cholinesterase and lipase are said to be reduced in the plasma and brain of vitamin E deficient rats [264] Hickman [265] reports that vitamin E does not inhibit lipoxidase but does inhibit chain and side reactions The other changes which occur as a result of a lack of vitamin E are a decrease in cholinesterase activity in the liver, brain, serum [372] and muscle [374] an increase in the cholesterol content of dystrophic muscles and the blood [177] and brain [157, 373]—though this has been denied for chicks [293]—and some increase in other lipoids in the muscles [373] but not in the heart or other organs [177] It is interesting to note that alpha tocopherol is said to raise the level of cholesterol and fatty acids in the blood of schizophrenics [375] The relation between vitamin E in the blood and cholesterol is described on p 601,

The Relation of Vitamin E to Pigmentation Martin and Moore in 1936 first observed a brown fluorescent discoloration of the uterus of rats deprived of vitamin E Later work by Martin and Moore [138], Moore and Wang [256], Barrie [141] and many others [257–260] has shown that this fluorescent discoloration is due to small yellow brown, acid fast, iron free insoluble granules within the muscle cells of the uterus The uterus however, is only the first tissue of the rat to be affected, nearly every other tissue becoming affected if the deficiency is sufficiently prolonged Thus in the rat pigment also appears ultimately in voluntary muscles, the diaphragm apart from the area round the œsophagus, the heart, kidneys, ovarian stroma and Graafian follicles [256] , and in the fallopian tubes, vagina, trabeculæ and capsule of the spleen, seminal vesicles, prostate, vas deferens ureter bronchi small intestine and the walls of the pulmonary and uterine blood vessels [257] , in the interstitial tissue of the testis and the motor ganglion cells of the cord and medulla [258] , and within the fat cells [259] The appearance of pigment in the lymph nodes is probably due to its transport there by the macrophages which can be seen in the affected tissues packed with the pigment [257, 259] The fœtal rat from a brown uterus remains unpigmented [256]

The pigmented uterus of the rat responds normally to drugs (p 607) The pigment does not appear, or only very slightly in the infantile uterus (p 607) and can be reduced by vitamin E together with a normal pregnancy [141] though vitamin E alone has no affect [138] In the mouse [111] the pigment is said to be only in the reticulo endothelial cells of all organs save the heart, liver and adrenal cortex, where it appears within the parenchymal cells In guinea pigs pigment has been reported in the testes [258] but not in dystrophic muscle [256] , in dystrophic calves no mention is made of pigment [131] while it appears to be readily produced in the hamster and cotton rat and dog [87] In the monkey [87] pigmentation of the smooth muscle of the blood vessels and intestine is very pronounced and less pronounced in the muscle of the urinary bladder gall bladder, bronchi,

Moore and Wang [256] from
cted pigment, believe it to be
oxidized and partially deaminated protein Most other workers [259, 260] however, hold that it is formed by the polymerization of the peroxides of poly unsaturated fatty acids, basing this belief on its histochemistry [260] and on the observation that the peroxide value of fat increases as the pigmentation increases [259 261] The pigment may be the same as the

"ceroid" pigment, thoroughly described by Endicott and Lillie [262] in regard to its histochemistry, which occurs in the livers of rats rendered cirrhotic by a deficiency of protein Since such cirrhosis producing diets generally have been deficient in vitamin E and when rich in vitamin E have been reported to cause cirrhosis without "ceroid" [258], it would seem very probable that "ceroid" and the pigment induced by lack of vitamin E are the same a probability increased by their very similar properties [260, 262] If they do differ slightly it could well be that this is only because the cell labouring under lack of vitamin E produces slightly different abnormal waste products according to whether there is too little protein or too much fat, in the diet There is also a second fat soluble pigment caused by lack of vitamin E about which nothing is known [259]

In human tissues "ceroid," with the distribution of the pigment found in vitamin E deficient animals, has been found in conditions where the absorption of vitamin E has probably been grossly impaired [56, 258] and where other changes also indicative of lack of vitamin E, such as muscular dystrophy, have occurred Pigments similar to or identical with ceroid have been found in many tissues—especially in atrophic testes and in association with hæmochromatosis [258]

The practical implications of this production of pigment by a dietetic deficiency are fascinating Old age is associated with the appearance of various pigments within the cells of the body. They appear to represent an accumulation within the cell of waste products which cannot be excreted —in fact "metabolic clinkers" It is possible that the necessity for sexual reproduction, for recurrently rebuilding the animal from a single cell, is that old cells become too choked with unexcretable pigments to live As lack of vitamin E is one reason for the accumulation of such pigments within the cells of animals and as a similar or identical pigment is found in many human tissues—especially when deprived of vitamin E—it well may be that a mild deficiency of vitamin E when prolonged for many years greatly increases the choking of the cells with "clinkers" and so is one of the reasons, and probably the only avoidable reason, for senile degeneration

Dental Depigmentation of the perpetually growing incisor teeth of rats deprived of vitamin E was first observed by Davies and Moore [266] in 1941, who later showed that piebald rats are more resistant to this bleaching than are albino rats [267] The pigment of the teeth is not a lipochrome, porphyrin or melanin but does contain inorganic iron in the ferric form [268] In the depigmented teeth iron is greatly reduced both in the enamel and dentine, while manganese is greatly increased, and magnesium is slightly increased in the enamel but decreased in the dentine, calcium and phosphorus are unaltered [268] The alkaline phosphatase activity in the enamel organ is not altered [269], as might have been expected from the rise in manganese Manganese, *nordihydroguaiaretic* acid [270] and protein [270, 272] give some protection against depigmentation, but iodine and copper and castration [270] and iron [268] give no protection, nor does lack of iron cause depigmentation [267] Pigmentation of fat and depigmentation of teeth do not always run parallel with each other [270]

Irving [271] has described changes in the incisors of rats which he believes only occur from lack of vitamin E In the centre of the middle third of the tooth there is a sudden premature and abnormal degeneration of all layers of the enamel organ which is replaced by fibrous tissue This is reminiscent of the depigmentation and damage to the enamel organ caused by lack of vitamin A (p 35)

The Relation of Vitamin E to the Blood and Blood Vessels Lack of vitamin E decreases the resistance of red blood cells to hæmolysis by dialuric acid (p 598) and several investigators have reported anæmia in deficient animals, but here the very low protein intake [272] or the very high cod liver oil intake [280] blur the picture Megakaryocytes are reported to be greatly

increased in the bone marrow with myeloblasts and myelocytes in the lymph nodes and early forms of granulocytes in the spleen [230] In dystrophic rabbits there is increased hæmoglobin formation [289] Anæmia cannot well be a major result of lack of vitamin E or it would have attracted far more attention than it has In monkeys lack of vitamin E causes a polymorpho leucocytosis [92] Vitamin E is said to be antithrombic *in vivo* at concentra tions normally present in the blood and so may help in preventing intra vascular clotting [281] this is supported by clinical work (p 658) The purpura caused in dogs by stilbœstrol is stated on account of very unconvinc ing work [283] to be prevented and cured by oral daily doses of 200 mg of vitamin E

A great and rapid increase in the collateral circulation round post traumatic venous thromboses in dogs and decreased inflammatory and degenerative changes in the walls of the veins is caused by 140 mg daily of vitamin E according to very excellent and well controlled work by Enria and Ferrero [282] Holman [285] reports that vitamin E in daily doses of 2 5 to 4 mg per kilo of body weight protects dogs on a high cod liver oil diet from the arterial lesions which develop after the kidneys have been damaged by agents such as uranium The arterial lesions are very like periarteritis nodosa and rheumatic arteritis consisting of œdema swelling fragmentation and ultimately necrosis of collagen with fibrin and intense leukocytic reactions In rabbits Manuel and others [84] state that vitamin E gives a high degree of protection against arteriosclerosis induced by cholesterol though Dam [286] denies this both for rabbits and chicks and Bruger [287] even found in rabbits given extra cholesterol that the cholesterol content of the aorta was raised by vitamin E In the rat [288] large doses of vitamin E are said to cause sclerotic patches and cholesterol deposits in the aorta even though no extra cholesterol is given in the diet lack of vitamin E on the other hand merely causes some fatty infiltration Clinical work is discussed on p 657

The Relation of Vitamin E to other Tissues Neoplasms Resistance to Infection and Poisons *Fur and Skin* The fur of rats on a diet low in vitamin E is reported to remain infantile and unusually white in the young [^ while in the adult it becomes coarse sparse seen in Fig 216 In young pigs the coat have often been reported in rats [138] and in dogs [59] We have often found wheat germ most valuable for the coats and health of dogs

Osseous Tissues Very soft skulls with little ossification have been observed in the suckling young of rats deprived of vitamin E by Barrie [143] and a similar condition in adult rats has been reported in a personal com munication by Wright Very high intakes of vitamin E cause decalcifica tion [191] The changes in the mineral content of the teeth of deficient rats also suggest that vitamin E plays an important part in mineral metabolism (p 622)

Eyes The eyes of adult vitamin E deficient rats have been said to be unduly protuberant by Demole and Pfaltz [294] and this has also been observed in the young by Lecoq and Isidor [230] Demole and Knapp [295] report that in adult rats a deficiency of vitamin E causes clouding and vascularization of the cornea keratoconus iridocyclitis opacities of the lens and serous retinal exudates Though such changes have never been reported in any of the vast numbers of rats which have been observed by other workers yet they are of interest in view of the clinical work reported on p 658 and the protuberant eyes seen in some children with muscular dystrophy (p 635)

Renal Degeneration Martin and Moore [138] have observed a slow progressive parenchymatous degeneration of the kidney unaccompanied by any inflammatory reaction or by any change in the blood vessels The

renal changes occur after deprivation of vitamin E for three or four months after ten months nearly all the convoluted tubules are destroyed so that it is surprising that the animal yet survives The degeneration starts in the cells lining the convoluted tubules which become granular and detached

FIG 217 Photomicrograph of normal kidney from a rat given a vitamin E concentrate

FIG 218 Photomicrograph of kidney from a rat on a diet deficient in vitamin E Note the extensive degeneration and detachment of the epithelial lining of the urinary tubules

from their basement membrane In time the degeneration may spread to the loops of Henle and the collecting tubules The glomeruli show little change (Figs 217 218)

Neoplasms Early work on the relation of vitamin E to neoplasms was complicated by lack of any pure preparation of vitamin E so that wheat germ oil had to be used Nothing but contradictory reports without any

clear cut results emerged from this period of research, though those who are interested will find references to it in the paper by Telford [296] and in the second edition of this book The current use, however, of pure alpha-
Telford [296] induced
, 5, 6 dibenzanthracene
s often and in smaller
numbers than mice given 2 mg of alpha tocopherol every other day But
there were only twenty four m
cent of these as against thirty
cysts which often broke down
so that probably much was i
best only suggests vitamin E may stimulate neoplastic growth, and it is contradicted by a report [297] that alpha-tocopherol causes regression of spontaneous mammary tumours in female mice, though the same report states that in male mice vitamin E may even stimulate the growth of tumours

Cater [32], investigating Rous sarcoma in chicks, has contributed the most important work on vitamin E and neoplasms He found that the largest tumours relative to the weight of the chick occurred in deficient chicks and the smallest tumours in chicks given large amounts of vitamin E This is important as it is usual for tumours to grow badly when the host is suffering from general malnutrition, such as inevitably occurred in the vitamin E deficient chicks Cater suggests that vitamin E acts as a controller of mesenchymal growth while Rous sarcoma produces a growth promoting substance Therefore the vitamin controls the exuberant growth caused by the sarcoma In support of this he reports that Rous sarcoma and lack of vitamin E both stimulate the activity of the preen gland and both cause a precocious development of the testes the gland and the testes finally atrophy as a result of this excessive stimulation This theory, though startling, is in harmony with the way in which the muscles in muscular dystrophy burn themselves away

Resistance to Infections and Poisons The work on vitamin E and infections [298–301] is too slight and indefinite to be worth considering The rôle played by some antibiotics and sulphonamides in giving partial protection against massive hepatic necrosis of dietetic origin is discussed on p 620

Poisons and vitamin E appear to be often intimately linked together Thus Daft and others [279], from well controlled work on young rats, have shown that succinylsulphathiazole accelerates the development of muscular dystrophy in vitamin E deficient animals, though the poorly absorbed sulphaguanidine has no such effect Meunier and Chenavier [302] found that while normally fed rabbits given di o cresyl succinate died within seven days from diarrhœa, loss of weight and paralysis, similar rabbits given 50 mg of alpha tocopherol completely recovered Akin to these observations are those of Hove [303] who found that carbon tetrachloride poisoning in rats on a low protein diet could be cured by vitamin E Hove states that the poisoned rats developed symptoms identical with those induced by lack of vitamin E The theory which best accounts for all the above observations is that vitamin E plays an important or, when protein is scarce (p 620), an essential part in destroying various poisons and is itself destroyed in the process It would be of great practical value to know what effect vitamin E has on the toxicity caused by flour treated, as it always is in England, with nitrogen trichloride or "agene"

VITAMIN E IN FOODS

There is startlingly little vitamin E in English food some 4 to 8 mg of alpha tocopherol would seem, from the food tables given later, to be the

average amount consumed daily in 1952. In the U.S.A. [43] about 14 mg. daily is the average while in the Netherlands [65] it is about 15 mg. The serious consequences of the poverty of English food in vitamin E are discussed on p. 631.

Bread should hold a pre-eminent place as a source of vitamin E. In the Netherlands [65] it provides more than one-third of the total, but in England [236] bread is to all intents valueless because it is made of eighty-one per cent. extraction home-milled flour and seventy-two per cent. extraction foreign flour, with potato flour when this is not too costly. The miserable bread made from this flour should have about 0·05 mg. per slice but the Government in its infinite lunacy continues to permit the agenizing of flour, and as this destroys about three-quarters of the vitamin E in the flour [237, 238] we are left with possibly 0·013 mg. in one slice. Since agene may be toxic—its use being banned in the U.S.A., France, Holland and Germany—and since vitamin E is probably essential for many of the detoxifying activities of the body (pp. 620, 625), the Government is indeed to be congratulated on managing at one and the same time to introduce a probable poison and remove the vitamin possibly essential for its destruction.

> "Bleached flour does more than Malthus can
> To sterilize the Englishman."

The best way by which the citizen can avoid eating this malnutritious flour and bread is by buying eighty-one per cent. extraction stone ground flour and making his own bread: a surprisingly easy occupation. The bread is a light cream in colour, contains the germ but no bran, and keeps excellently. Being as bland as white bread, it is suitable for patients with gastritis or "weak digestions." Some "brown breads" contain the germ, but often this has been removed during milling, specially treated, and then returned again to the flour. Such treatment may well destroy some or all of its vitamins. There are no reasons against using stone ground flour in domestic cookery, since it is as good as white flour for pastry and soufflés.

A further great advantage of stone ground flour is that it contains less phytic acid than brown flours, so that there is less danger of calcium, iron, and the other minerals present in the diet forming insoluble phytates in the gut with their consequent loss to the body (p. 527).

Brown rice, oatmeal and indeed all the cereals from which the germ has not been removed are excellent sources of vitamin E, but too often porridge is replaced by facilely prepared breakfast foods of no value whatsoever.

Green vegetables are a good source of vitamin E and so are some root vegetables, while potatoes are almost valueless: values however, as can be seen from the food tables, vary greatly. They appear to be much higher in the Netherlands [65] than in the U.S.A. [43], while the values of English vegetables are unknown. Possibly, however, the absorption of vitamin E from vegetables is very poor [65]. *Fruit*, were it eaten in large amounts, would be a valuable source, and rose hips are very rich in vitamin E [illegible] but probably the seeds and not the pulp contain th[illegible]p jam will not add to the consumption [illegible]

Meat could be a good sou[illegible] chilling or freezing (p. 627) appears to destroy vi[illegible] E, and as most meat eaten in England has been frozen for weeks or months and in any case is eaten in minute amounts, the contribution of meat to our intake of vitamin E is negligible.

Fish, or at least sardines, cod's roe and haddock, is an excellent source.

Dairy produce depends for its value on the diet of the cows and hens. Milk, and so butter, from pasture fed cows is richer than milk from those stall fed, and the milk from Guernsey's is about three times as rich as the poor milk of Holsteins [239]. The colour of the milk is a good guide to the amount of vitamin E it contains, since carotene and vitamin E fluctuate together [239]. Eggs reflect the diet of the hen, containing per 100 grams as little as

0 10 mg or as much as 4 0 mg [62] it is probable the colour of the yolk is again a guide to the value of the egg

Margarine *may* be an excellent source depending on what oils have been used in its manufacture

Effect of Rancidity, Staleness, Storage and Cooking Rancidity rapidly destroys vitamin E (p 615) This is probably of importance in human diets Most human foods containing fat are eaten stale and even if they do not smell rancid may well have lost all their vitamin E since this will be destroyed before frank rancidity can develop Conversely an off flavour in meat poultry and milk is often a sign that the animals lacked vitamin E in their diets [239 240 241]

Storage of meat and presumably of all foods except whole grains destroys vitamin E even at low temperatures [242 243] For instance Dju and others [243] report that twenty two per cent of the vitamin E in animal fat is destroyed after only one month s storage at − 20 °C thirty nine per cent after ten weeks and forty six per cent after twenty weeks For the English whose meat and fish and even vegetables are often only chilled at − 2° C or frozen at only − 9° C for many weeks before they rest for days in the domestic refrigerator the loss of vitamin E must be very high indeed

Domestic cooking probably causes no appreciable loss though little vitamin E remains in fat recurrently used for deep fat frying [43] There is some evidence that dried and pasteurized milks may contain no vitamin E [244] so artificially fed babies may receive inadequate supplies From the single morsel of evidence that vitamin E is not destroyed in the canning of cod s roe [245] it seems possible that canning is not deleterious

Preparations of Vitamin E Available for Medical Use Vitamin E may be given as the pure natural or synthetic tocopherols as wheat germ oil or as wheat germ The latter is by far the best when only small amounts are required

Pure Tocopherols The relative values of the different natural and synthetic tocopherols have been discussed on p 594 so here it is only necessary to point out that as yet there are no preparations suitable for injection (p 593) It is also important to remember that alpha tocopherol is so much more active than the other tocopherols that the content of the latter in any mixtures can be ignored The advantage of the pure tocopherols is that enormous oral doses may be given though most of such doses are excreted (p 599) Probably it is wise to give tablets of the tocopherols

ieal as they are fat soluble and probably

600)

in wheat germ oil is unusual in being composed of about seventy per cent of alpha tocopherol and thirty per cent of beta tocopherol the latter is only present in very small amounts in oils and foods not derived from wheat but as it has a low biological potency compared to alpha tocopherol (p 594) it would appear to have no particular

absorbed (p 600) Wheat germ oil has been shown [246] by work which appears to have been well controlled to have a marked stimulating effect on the growth of the ovaries uterus and adrenals of rats If this endocrine effect should be confirmed it would raise very complicated problems not only about the medicinal use of wheat germ oil but also might still further emphasize the importance of leaving the germ in flour and bread

Whole Wheat Germ This is undoubtedly the best method of giving vitamin E A the vitamin B complex and many ot h have a marked effect in enhancing xidants in the germ give considerable protection to vitamin E against destruction by rancid fats

during digestion Further the germ keeps excellently if a reliable preparation is used germ obtained from mills however may become rancid in a couple of days It is difficult to understand why wheat germ oil should ever be

t germ It is certain the oil cannot contain

t germ and it is equally certain that much

without doubt lost in as far as the vitamin B

complex and minerals are concerned

The trace elements whose great importance in relation to the vitamins is only slowly being realized should also be remembered when considering the value of wheat germ since it is one of their richest sources notably for zinc [247] and manganese [248] The trace elements are intimately connected with the proper use by the body of the vitamins [249 250 251] and so should be supplied when treating deficiency diseases especially as the distribution of the trace elements in foods largely follows that of the vitamins so that when there is a deficiency of the latter there is also most probably a deficiency of the former [247 252] The use of whole wheat germ ensures that the value of the vitamins it contains is not limited by the absence of those

ogical activity of the vitamins

vitamin preparations

m wheat germ oil and the

vitamin B complex should then be given instead [10 253] and this combination may be used to give children a short rest from wheat germ during prolonged treatment

Bicknell finds that wheat germ very occasionally causes an urticarial rash in children who are being treated for muscular dystrophy It appears like other forms of urticaria most often after the child has had a hot bath or is warm in bed After a few months it disappears, in the meantime it is best treated with the usual lotions and not with drugs Donovan [254] has also seen a similar rash in a boy with muscular dystrophy who was taking wheat germ Stone [253] using wheat germ oil for children with muscular dystrophy and Shute [7] using it for adults with sterility or threatened abortions have reported occasional skin rashes Newman [255] using wheat germ and

Apart from these rar

tolerated and do not c

Vitamin E should not be given close to a meal which has contained any food which may be on the verge of rancidity In children cod liver oil and cod liver oil concentrates should be given at different meals to the wheat germ All foods which are not fresh and all cheese should be avoided

AMOUNTS OF VITAMIN E IN FOODS

In the following table—which is largely derived from those of Emmerie and Engel [33] and Harris Quaife and Swanson [43]—all the values for vitamin E are based on chemical estimations since no accurate biological estimations have been performed owing to the enormous labour these involve Of the various tocopherols which together make up the vitamin E activity of a food alpha tocopherol is by far the most biologically active (p 594) so only the alpha tocopherol and total tocopherol values are given The difference between these values is for most foods made up almost entirely of gamma and delta tocopherols the activity of these is so slight (p 594) it may be ignored Wheat and its products however are exceptional in having about thirty per cent of their tocopherols as beta tocopherol and sixty five per cent as alpha tocopherol the former has roughly one third the value of the latter Soya beans are the opposite of wheat the almost inert delta tocopherol forming about thirty per cent of the total tocopherols [13] and alpha tocopherol about ten per cent Only the total tocopherol content of some foods is known this has been given though it is of scant help in

estimating the vitamin E value of a food since the active alpha tocopherol may form as much as seventy per cent or as little as ten per cent of the total tocopherols

Food	Milligrams in 100 grams or roughly 3½ ounces	
	α Tocopherol	Total Tocopherols
Cereals		
Barley	? 1 6–2 6	3 2–5 2
Agenized Germ	0 4 0 6	0 8 1 3
		14
"Bemax"	? 14	28
Bread, 100% extraction flour	? 0 75	1 3
80% extraction flour	? 0 12	0 23
English agenized (p 626)	? 0 03	? 0 06
Cassava (East Indies)	—	0 2
Flour (wheat) whole	? 1 5	2 2
80% extraction	? 0 84	1 2
English agenized (p 626)	? 0 21	? 0 3
Groats	—	1 2
rolled	—	1 5
Cornmeal yellow	0 84	1 7
Oatmeal	1 94	2 1
Panicum viride	—	4 0
Rice, brown	1 2	2 4
polished	0 35	0 57
bran	—	3 0
Rye	? 1 1 1 7	2 2–3 5
agenized	? 0 3–0 4	0 6 0 9
Spaghetti	? 0 6	1 20
Wheat germ	18 9	27
Dairy Products		
Butter, Dutch, U S A	—	2 1 3 5
Cheese, American	—	1 00
10% fat	—	0 3
20% fat	—	0 6
Eggs (p 626)	1 2	1 4
Milk (p 626), cow's	—	0 02 1 2
summer	—	0 17
winter	—	0 08–0 15
pasteurized (p 627)	—	? None
powdered (p 627)	—	0 3–0 5
skim (p 627)	—	0 05
ewe's milk	—	1 5
colostrum	—	4 7
goat's milk	—	1 4
colostrum	—	5 9
sow's colostrum	—	18 6
woman's early	—	0 13–3 6
late	—	0 11 1 64
Fish		
Cod, liver oil (fresh)	—	13–14
roe (fresh or canned)	—	5 25–7 5
Haddock	0 35	0 39
Herring		0 5
Liver oils (pp 595 616)	—	?
Mackerel	—	1 8
Salmon	—	1 6
Sardines	—	4 5
Shark liver oil	—	10 ? 40

Food	Milligrams in 100 grams or roughly 3½ ounces	
	α Tocopherol	Total Tocopherols
Fruits		
Apples	0 72	0 74
Banana	0 37	0 4
Capsicum	—	2 4
Coconut	—	0 2
Grapefruit	0 25	0 26
Oranges	0 23	0 24
Meat (p. 627)		
Beef, liver	1 4	1 4
steak	0 47	0 63
brain	—	1 2–2 3
Chicken	0 21	0 25
Lamb chops	0 62	0 77
Pig, bacon	0 41	0 53
pork chops	0 63	0 71
lard	2 3	2 7
Oils and Fats, Vegetable		
	24	48
Arachis or peanut oil	11	22
	15 6–21 6	26–36
Beechnut	—	100
Cocoa fat	—	12 5
Coconut oil	3 6	—
old	—	3 6–5
hydrogenated	—	0
	7	87
Corn oil	10–13	90–102
		250
Cottonseed oil	43	86
refined	56	90
		60
Linseed oil	—	23
Maize oil	—	70–250
		—
Margarine	—	?
Olive oil	—	3–8
Palm oil	30	56
red	—	110
Rice bran oil	55	91
Sesame oil	—	5
	11 9	40
Soya bean oil	10	168
	—	140
	9–12	92–120
Wheat germ oil, crude	—	100–420
medicinal	—	320
Vegetables		
Beans, dried navy	0 1	3 6
kidney	—	1 2
white	—	4
Beetroot	—	0 2
Brussels sprouts	—	1 7
Cabbage	0 06	0 11
red	—	0 2
white	—	0 7
Carica papaya, leaves	—	36

Food	Milligrams in 100 grams or roughly 3½ ounces	
	a Tocopherol	Total Tocopherols
egetables—continued		
Carrots	0 45	0 45
	—	1 5
Celery	0 46	0 48
		2 6
Endive	—	2 0
Belgium	—	0 2
Ipomœa leaves	—	8 1–11 8
Kale	—	8 0
Leek	—	1 9
Lettuce	0 29	0 43 0 54
Onions	0 21	0 26
Parsley	—	5 5
Peas green	0 1	2 1
	—	5 1 6 4
Potatoes boiled	—	0 1
white, peeled	—	0 06
sweet	4 0	4 0
Radishes black	—	0 04
Scorzonera	—	0 6
Soybean	—	18 8
Spinach	—	1 7
Tomatoes	0 27	0 36
Turnip	—	0 02
greens	2 24	2 3
arious		
Chocolate unsweetened	5 3	11 1
Cocoa powder	—	3 1
Fungi	—	0
Peanuts	4 6	9 3
	—	6 3 11 9
Yeast	—	0

HUMAN REQUIREMENTS OF VITAMIN E

Nothing is accurately known of the human requirements of vitamin E though from the figures given in the preceding table many of the English must consume less than 5 mg a day yet still continue in what in England is regarded as good health Indeed vitamin E has been considered to be of no importance in human nutrition But it is fantastic to believe that while mice and monkeys ducks and dogs beetles and barrows all need vitamin E yet a beneficent all wise biological providence has excused man from this irksome necessity In most animals complete deprivation of vitamin E leads to rapid and overt illness but when some small amounts of vitamin E are given in the food over long periods—as they are in the food of the English—the symptoms are covert merely a disturbance of the reproductive system in old rats (p 607) or slight myocardial damage in the monkey (p 615) or apparently complete health in the cow for years until there is sudden death (p 606) There is also the probability (p 621) that insufficient vitamin E is one of the reasons why senility pigments or metabolic clinkers collect in the body s cells over the long course of the years first to damp and finally to smother the bright flame of life

It is impossible to believe that vitamin E is not essential for man but it well may be that the symptoms of a prolonged mild deficiency only show

themselves after many years, and then are so blurred by the changes of senility which they themselves helped to produce, that they are difficult, impossible, to recognize It is grievous that our food loses so much of what may be an essential protection against senility merely because the vision of science is just as long as the life of a rat

DISEASES DUE TO A DEFICIENCY OF VITAMIN E
ABORTION AND OTHER SEXUAL DISORDERS

The tendency for the birthrate to decline among industrial or "civilized" communities is a serious problem There are probably many causes for this among which a deficiency of vitamin E
ance Drummond and W
have drawn attention to
and the fall in our consum
of white flour from whicl
has pointed out that the agricultural labourer of to day has apparently a better diet than he did fifty years ago, but he eats white flour where he used
thus low fertility
are both benefits
to be efficiently
used by most labourers

Hogbin [305] mentions reports that the spread of refined foods low in vitamin E has been one of the causes of the falling fertility in some Pacific Islands, while Young [13] suggests that racial fertility is connected with diet, and that though the number of abortions due to a faulty diet is unknown "we may, however, safely assume that it constitutes a considerable proportion of spontaneous cases" The importance of abortion, as apart from a decline in fertility, is shown by the fact that in England and America one fifth of all pregn
e abortions
from all
The level
of vitam
p 601, and
experime
0

Recurrent Abortion. Vitamin E has never gained much popularity with gynæcologists in the treatment of recurrent abortion Browne [7, 307] for instance, has pointed out that as good results are obtained with endocrine preparations, simple rules of conduct, or indeed with no treatment save placebos This rather hopeless attitude towards the problem may be criticized firstly on the grounds that Bacharach's [308] statistical examination of the published clinical results show quite definitely that vitamin E is of value in recurrent abortions Secondly, because one form of treatment gives good results is no reason for doubting the value of another In those women who tend to abort there may well be several mildly injurious factors—endocrine, dietetic, psychological—which added together terminate the pregnancy The removal of any one will enable the pregnancy to proceed The relation of abortion to the level of vitamin E in the blood is discussed on p 602

In treatment wheat germ oil has generally been used as the source of vitamin E should the endocrine activity of wheat germ oil (p 627) be confirmed it is possible that its vitamin E accounts for only a part of its effect

Vogt Moller [8] in 1931 first used wheat germ oil in the treatment of habitual abortion He only reported two cases treatment was successful in both though one woman had previously had four successive abortions and the other five In the next five years he treated seventy two women with no demonstrable reason for their recurrent abortions with 3 grams of wheat germ oil daily Fifty five living children were born, all of whom were well developed In 1939 he summed up his own experiences and

reports in twelve other papers Since 1936 my evidence from such cases has increased considerably Taken as a whole favourable results were obtained in about eighty per cent of cases Many investigations have confirmed my observations The records of treated cases of habitual abortion now amount to some hundreds with a mean value of seventy five to eighty per cent favourable results [7]

Currie [7] in 1939 reported his results from giving about 6 mg of tocopherol daily in the form of concentrated wheat germ oil The length of treatment varied from three to thirty two weeks and was often not begun until the middle weeks of pregnancy Treatment with luteinizing hormone did not give such good results In all eighty one women were treated who had had two hundred and seventy four previous pregnancies of which only forty seven had gone to term With treatment the next eighty one pregnancies resulted in sixty two viable infants being born Isles [7] using the same dose of vitamin E as Currie treated eight women who had had only five successful pregnancies out of twenty nine with complete success eight viable infants being born

Watson [309] had successful results in twenty one of twenty eight women who had had two or more abortions and in eight of nine who had had one previous abortion Lubin and Waltman s figures [319] from giving 3 mg of synthetic vitamin E daily were respectively five out of seven and eight out of ten

Malpas [310] however, criticized the value of wheat germ oil on the grounds that it gave no better results than those which would have been expected had no treatment been given From his own investigations and those of others he concluded that eighteen per cent of all pregnancies end in abortion and of these abortions only one is due to recurrent causes From this he deduced that in one hundred pregnant women treatment could at best only lead to eighty three live births and better results than this proved too much But why he should assume that lack of vitamin E could only cause recurrent abortions and not contribute to the seventeen per cent of non recurrent abortions is obscure Many reasons might lead to a transitory deficiency of vitamin E which only was present during one conception and so treatment with vitamin E might well lead to more than eighty three per cent of liv[illegible]
that lack of vitamin E may [illegible]
once for no obvious reas[illegible]
seventeen per cent of all pregnancies must end in abortion he states that the expectation of living children in untreated cases of recurrent abortion is after two successive abortions sixty two per cent after three twenty seven per cent and after four six per cent

Bacharach [308] has examined the figures of Malpas and applied them in a slightly modified form to the published results of the treatment of habitual abortion He points out that treatment has given far better results than would be expected from Malpas s figures were it valueless In fact the probability of chance alone accounting for the apparent success of treatment is after four or more consecutive abortions 1 in 10^{40} after three or more 1 in 10^{10} after two or more 1 in 200 This analysis of Bacharach s appears definitely to confirm the value of wheat germ oil in the treatment of recurrent abortions

Threatened Abortion and Toxæmias of Pregnancy Shute [311] observed that when rats became sterile from lack of vitamin E the resistance of their blood serum to proteolysis was increased Treatment with vitamin E removed this antiproteolytic factor from the blood He then investigated women who were threatening to abort and found that their serum was similar to that of vitamin E deficient rats in its resistance to proteolysis Giving these women vitamin E returned the blood serum to normal

Work by Jeffcoate [315] Shute [312] and others suggests that this

antiproteolytic factor is of an œstrogenic nature Its presence hinders the proteolytic erosion of the placental villi into the uterine wall This in turn leads to a weak attachment of the placenta and so to termination of the pregnancy.

From all these observations Shute [311] was lead to believe that "vitamin E and œstrin, or a substance very much like it, exist in a sort of equilibrium during pregnancy If there is too much of the œstrin like substance the pregnancy is interrupted An excess of vitamin E appears to have no effect on the pregnancy." Therefore in cases of threatened abortion with premature partial separation of the placenta and a high antiproteolytic factor in the blood vitamin E should be of value in treatment Shute has treated a considerable number of such cases with wheat germ oil, and claims in one series success in sixty-eight per cent of one hundred and eighteen cases [7] Young [13] also found wheat germ oil of value in cases of threatened abortion, and so have others using synthetic vitamin E [319] Again it will be noticed that wheat germ oil with its possible endocrine activity (p 627) and not pure vitamin E has been mainly used in treatment

An objection raised to preventing threatened abortion occurring is that it may be a sign of an abnormal fœtus which would be better dead But a study of all the available reports shows that only five out of eighty nine children born after a threatened abortion had congenital anomalies [7]

Shute [7] emphasizes that women should have their blood examined for the antiproteolytic factor early in pregnancy. If it is high they will require wheat germ oil throughout the whole pregnancy If this is not continued or is given in inadequate amounts toxæmia and premature placental detachment will occur later in the pregnancy, such as was observed by Young [13], who thought vitamin E given during pregnancy might unearth a later toxæmia

Pregnancy toxæmias may be divided, according to Shute [313], into those produced by too little vitamin E and those produced by too little œstrogen In the former type there is a raised blood pressure, œdema and albuminuria, and premature detachment of the placenta which gives rise to the name "hæmorrhagic toxæmia" This type responds to treatment with vitamin E The second type is the true eclamptic with low œstrogens, and must on no account be given vitamin E, as this further depresses the œstrogens and so causes convulsions Instead œstrogen therapy is required, though too much will convert the eclamptic to the hæmorrhagic type

The amount of wheat germ oil advocated by Shute [7] is large and should be supplemented with the vitamin B complex. An initial dose of 1½ ounces of fresh oil are given, and then 1–2 drachms daily till the end of pregnancy More may be needed to control the symptoms, especially towards the end of pregnancy and in patients with hypothyroidism, who tend to have high blood œstrogens In the summer the higher content of vitamin E in the food decreases the amount of oil required

Krieger [317], however, after very careful work could not confirm the presence of Shute's antiproteolytic factor, in the blood serum of cases of abortion premature labour, accidental hæmorrhage or normal pregnancy Cuthbertson and Drummond [316], using a slight modification of Shute's test [318] for the susceptibility of blood serum to proteolysis, failed to find any difference in the blood of rats reared on vitamin E deficient diets, normal diets, or diets rich in vitamin E These authors also criticize the biochemical theory of the test Drummond, Noble, and Wright [151] hold that Shute is wrong in believing that lack of vitamin E causes an excess of œstrogens in the blood chiefly because if this were so lack of vitamin E would produce symptoms of an œstrogenic excess in animals, such as failure to conceive, irregular œstrus, and testicular changes unlike those observed from a deficiency of vitamin E Shute [314] has answered these objections to his test and theory by saying his test was incorrectly carried out, and that the

presence of small amounts of œstrogens in the blood of animals need not have the effects Drummond and his collaborators mentioned In our opinion Shute's test is valueless.

E has been used, chiefly by German
itened abortion One possible reason
this dual therapy is that the vitamin
protects the hormone from destruction in the body (p 594) Winkler [320] reports that 30 mg of alpha tocopherol daily raises the low urinary excretion of pregnandiol which he has observed in cases of threatened abortion He and Bach [321] advise giving progesterone with the vitamin E during the first five days of treatment in order to tide over the period before the vitamin has had time to stimulate the corpus luteum In seventy four untreated cases there were twenty four abortions, in twenty seven cases treated with progesterone there were five abortions, in ten cases treated with 30 mg of alpha tocopherol there was one abortion, and in sixteen cases treated with both the hormone and the vitamin there were two abortions Schafer [322] successfully treated forty-five out of fifty three cases with vitamin E alone or combined with progesterone He does not state what dose of vitamin was given Silbernagel and Patterson [412] gave a wheat germ oil concentrate to 825 women, starting between the third and sixteenth week of pregnancy using as controls 1,973 untreated women The respective results for the untreated and treated were, in percentages, threatened abortion 16 6 and 10 , abortion 15 and 3 , prematurity 7 1 and 3 7 , toxæmia 10 and 2 1 , stillbirths 2 3 and 0 4

Primary Sterility. In both men and women primary sterility is probably not cured by vitamin E This is not surprising since deprivation of vitamin E causes an irreversible testicular change in male animals, while in female animals vitamin E is not necessary for conception (pp 606, 610) Farris [323] found no change in the sperm count of fertile and subfertile men treated with vitamin E and it has been suggested that such treatment may decrease the essential hyaluronidase in the semen (p 597) Some human testes apparently show degenerative changes due to lack of vitamin E, especially in those conditions such as the steatorrhœas where there is probably prolonged impaired absorption (p 600) In such conditions prophylactic treatment before degeneration has occurred should be of value

The Menopause. Menopausal flushes may be greatly benefited by vitamin E Thus McLaren [324] in an excellent paper on the treatment of forty seven severe cases with 500 mg daily of synthetic alpha tocopheryl acetate, states that in about five weeks, on an average, there was complete cure in twenty three cases, marked improvement in seven cases and no benefit in seventeen cases the latter did not respond to still heavier doses of vitamin E but did respond to stilbœstrol A year later of seventeen cases who had been cured by vitamin E, twelve had relapsed A careful and well controlled investigation by Finkler [325] shows that out of sixty six women excellent results were obtained in thirty-one, fair in sixteen and none in nineteen Only 30 mg daily of alpha tocopheryl acetate was used over periods of ten days to seven months No toxic effects were noticed by either of the above authors and they emphasize the value of vitamin E for these frequent cases for whom
ithors report that there are no changes
aren found a gradual improvement in
il lesions

MUSCULAR DYSTROPHY (PSEUDOHYPERTROPHIC MUSCULAR DYSTROPHY · PROGRESSIVE MUSCULAR DYSTROPHY : PRIMARY MYOPATHY)

Muscular dystrophy is probably caused by an inability of the muscles to use vitamin E This is thought to be so because —

(*a*) The peculiar muscular degeneration of muscular dystrophy in animals is caused and is *only* caused by lack of vitamin E [225]

(*b*) Human muscular dystrophy shows identically the same peculiar degeneration

(*c*) Muscular dystrophy can be induced in the monkey and in all other animals (p 605)

(*d*) Typical early muscular dystrophy has been found in the muscles of severely malnourished children [326] and in adults suffering from chronic intestinal conditions where absorption of vitamin E is presumably impaired (p 600)

(*e*) Muscular hypotonia in children may be remedied by vitamin E (p 647)

(*f*) An analogous failure to use a fat soluble vitamin is seen in children and adults suffering from "resistant rickets" (p 562) This too, like muscular dystrophy, may be familial

(*g*) A superabundance of vitamin E may check or even improve the muscular degeneration (p 643)

The reason why the muscles are unable to use vitamin E is obscure It is not due to a simple dietetic deficiency or a failure of absorption, since the diets of many dystrophic children have always been excellent and the level of vitamin in the blood is normal (p 602) Milhorat and Bartels [327] in 1945 suggested that during intestinal absorption vitamin E was acted upon by an enzyme and converted into the form which probably as a compound with inositol is active in the body Bicknell [328] in 1949 failed to confirm this theory He fed children who had not responded to wheat germ alone, with a mixture of wheat germ, wheat germ oil, synthetic alpha tocopheryl acetate inositol and various preparations of dried hog's stomach or dried duodenum or dried heart muscle There was no change in the creatine and creatinine excretions measured daily for several months This of course did not disprove the theory, but only showed that if such an enzyme existed it was not present in the preparations used or was not active under the conditions of the experiment

The lack of a second vitamin or some common constituent of food is not the reason for the failure of the muscles to use vitamin E Bicknell has given large doses of every available vitamin, together with vitamin E, without any extra benefit One boy had his daily creatine and creatin excretion measured almost continuously for two years while his diet was accurately recorded But no relation was found between the customary violent fluctuations in excretion and any article of his very excellent, varied and fresh diet In passing it is interesting to note that in one boy on delta tocopherol both the daily creatine and creatinine excretions were increased so that the two curves remained in the same relative positions to each other but at a higher level

We are, therefore, left with a strong logical case for believing that the key to the cure of muscular dystrophy is vitamin E, but we have as yet no knowledge of how to fit the key to the lock

History Muscular dystrophy was first separated from other forms of paralysis by Duchenne of Boulogne, in 1861 The three types of muscular dystrophy generally recognized are the common pseudohypertrophic type found in children, which was first described fully by Duchenne [329] in 1868 the juvenile or scapulo humeral form of Erb first recognized by him [331] in 1884, and the facio scapulo humeral type which bears the name of Londouzy and Dejerine [330] from their account of it in 1884 Intermediate forms are seen, and it must be stressed that all forms of dystrophy are only different manifestations of the same disease The part which heredity plays has been exhaustively studied by Bell [332]

Pseudohypertrophic Muscular Dystrophy This is the most common form of dystrophy seen in England, though it appears to be less common than the others in the United States judging by the relative numbers reported in

recent papers. It occurs in all European countries and also in India, Ceylon and Japan, and there seems no reason to suppose any race is exempt

Boys are affected far more often than girls. In the latter the condition tends to start later, often at the age of puberty, and to progress more slowly (Fig. 219)

A familial tendency is very marked, so that it is common to find in one family two or three brothers in different stages of the disease Generally the girls are spared even when their brothers are not, and older boys seldom develop the disease after their younger brothers have done so It is, in fact, the boys born after the one who develops dystrophy who require careful watching Heredity appears to play no part in the disease, possibly because those affected have in the past died before the age of marriage

The mothers of boys with muscular dystrophy especially when the condition has apparently been present from birth, often give a history suggesting that during pregnancy and lactation they themselves were deficient in vitamin E. Thus it is not uncommon to find that before the dystrophic infant was born there were recurrent abortions, and recently two mothers attributed the dystrophy of sons born in the middle of long and normal families to starvation during these particular pregnancies Prolonged nausea and vomiting has also been mentioned by mothers as a possible reason The number of cases investigated, however, is too small to make these observations more than suggestive

The age of onset is commonly between five and ten, but many children give a history of crawling late and never walking properly, while others, especially girls, may show the first symptoms at puberty or even later In these late cases the condition tends to be less typical and to progress more slowly.

Infections often appear to unmask or emphasize the weakness so that the disease is ascribed to such complaints as scarlet fever or pneumonia

The important changes occurring during the disease are increasing muscular weakness, increasing contractures, and a rare primary osseous atrophy.

Muscular Weakness. The picture of the disease is unique It is that of a child fighting in an unsympathetic world against an increasing but unrecognized weakness of his legs It is often surprising how weak the child becomes before he is taken to his doctor Boys at school may be blamed for months for being lazy and clumsy before any medical advice is sought Usually the earliest symptoms are slowness in running, frequent falls so that the knees are never free from bruises, and hesitancy about going up, and especially down, stairs Careful use is made of the banisters, and the children go down one foot at a time In some children the frequent falls cause fear of slippery floors and loose mats

If the weakness first shows itself in the lumbar muscles, and those joining the pelvis to the thighs, the typical low lumbar lordosis and protuberant stomach will appear The waddling wide based gait will also be seen as the child dips from side to side in his painful efforts to overcome his inability to lift up his legs by swinging them outward and forward with each step The weak dorsiflexors, evertors and external rotators of the feet cause the child to trip over his own dragging turned-in toes

When the child falls down he has difficulty in getting up again, so that he "climbs up himself" This way of getting up is often thought to be pathognomonic of muscular dystrophy, but it is displayed in all cases where the muscles of the lower trunk and legs are weak If the child is laid on his back and told to get up he first rolls over on to his stomach Then he gets into a crawling position and slowly walks his hands backward toward his toes at the same time trying to straighten his knees and hips The latter, however, he cannot do without the help given by his arms as he walks his hands up his legs (Fig 224)

(a) The peculiar muscular degeneration of muscular dystrophy in animals is caused and is *only* caused by lack of vitamin E [225]

(b) Human muscular dystrophy shows identically the same peculiar degeneration

(c) Muscular dystrophy can be induced in the monkey and in all other animals (p 605)

(d) Typical early muscular dystrophy has been found in the muscles of severely malnourished children [326] and in adults suffering from chronic intestinal conditions where absorption of vitamin E is presumably impaired (p 600)

(e) Muscular hypotonia in children may be remedied by vitamin E (p 647)

(f) An analogous failure to use a fat soluble vitamin is seen in children and adults suffering from resistant rickets (p 562) This too like muscular dystrophy may be familial

(g) A superabundance of vitamin E may check or even improve the muscular degeneration (p 643)

The reason why the muscles are unable to use vitamin E is obscure It is not due to a simple dietetic deficiency or a failure of absorption since the diets of many dystrophic children have always been excellent and the level of vitamin in the blood is normal (p 602) Milhorat and Bartels [327] in 1945 suggested that during intestinal absorption vitamin E was acted upon by an enzyme and converted into the form which probably as a compound with inositol is active in the body Bicknell [328] in 1949 failed to confirm this theory He fed children who had not responded to wheat germ alone with a mixture of wheat germ wheat germ oil synthetic alpha tocopheryl acetate inositol and various preparations of dried hog s stomach or dried duodenum or dried heart muscle There was no change in the creatine and creatinine excretions measured daily for several months This of course did not disprove the theory but only showed that if such an enzyme existed it was not present in the preparations used or was not active under the conditions of the experiment

The lack of a second vitamin or some common constituent of food is not the reason for the failure of the muscles to use vitamin E Bicknell has given large doses of every available vitamin together with vitamin E without any extra benefit One boy had his daily creatine and creatin excretion measured almost continuously for two years while his diet was accurately recorded But no relation was found between the customary violent fluctuations in excretion and any article of his very excellent varied and fresh diet In passing it is interesting to note that in one boy on delta tocopherol both the daily creatine and creatinine excretions were increased so that the two curves remained in the same relative positions to each other but at a higher level

We are therefore left with a strong logical case for believing that the key to the cure of muscular dystrophy is vitamin E but we have as yet no knowledge of how to fit the key to the lock

History Muscular dystrophy was first separated from other forms of paralysis by Duchenne of Boulogne in 1861 The three types of muscular dystrophy generally recognized are the common pseudohypertrophic type found in children which was first described fully by Duchenne [329] in 1868 the juvenile or scapulo humeral form of Erb first recognized by him [331] in 1884 and the facio scapulo humeral type which bears the name of Londouzy and Dejerine [330] from their account of it in 1884 Intermediate forms are seen and it must be stressed that all forms of dystrophy are only different manifestations of the same disease The part which heredity plays has been exhaustively studied by Bell [332]

Pseudohypertrophic Muscular Dystrophy This is the most common form of dystrophy seen in England though it appears to be less common than the others in the United States judging by the relative numbers reported in

recent papers It occurs in all European countries and also in India, Ceylon and Japan and there seems no reason to suppose any race is exempt

Boys are affected far more often than girls In the latter the condition tends to start later, often at the age of puberty, and to progress more slowly (Fig 219)

A familial tendency is very marked so that it is common to find in one family two or three brothers in different stages of the disease Generally the girls are spared even when their brothers are not and older boys seldom develop the disease after their younger brothers have done so It is in fact the boys born after the one who develops dystrophy who require careful watching Heredity appears to play no part in the disease possibly because those affected have in the past died before the age of marriage

The mothers of boys with muscular dystrophy especially when the condition has apparently been present from birth often give a history suggesting that during pregnancy and lactation they themselves were deficient in vitamin E Thus it is not uncommon to find that before the dystrophic infant was born there were recurrent abortions and recently two mothers attributed the dystrophy of sons born in the middle of long and normal families to starvation during these particular pregnancies Prolonged nausea and vomiting has also been mentioned by mothers as a possible reason The number of cases investigated however is too small to make these observations more than suggestive

The age of onset is commonly between five and ten but many children give a history of crawling late and never walking properly while others especially girls, may show the first symptoms at puberty or even later In these late cases the condition tends to be less typical and to progress more slowly

Infections often appear to unmask or emphasize the weakness so that the disease is ascribed to such complaints as scarlet fever or pneumonia

The important changes occurring during the disease are increasing muscular weakness, increasing contractures and a rare primary osseous atrophy

Muscular Weakness The picture of the disease is unique It is that of a child fighting in an unsympathetic world against an increasing but unrecognized weakness of his legs It is often surprising how weak the child becomes before he is taken to his doctor Boys at school may be blamed for months for being lazy and clumsy before any medical advice is sought Usually the earliest symptoms are slowness in running frequent falls so that the knees are never free from bruises and hesitancy about going up, and especially down stairs Careful use is made of the banisters and the children

me children the frequent falls cause fear

in the lumbar muscles and those joining the pelvis to the thighs the typical low lumbar lordosis and protuberant stomach will appear The waddling wide based gait will also be seen as the child dips from side to side in his painful efforts to overcome his inability to lift up his legs by swinging them outward and forward with each step The weak dorsiflexors evertors and external rotators of the feet cause the child to trip over his own dragging turned in toes

When the child falls down he has difficulty in getting up again so that he "climbs up himself" This way of getting up is often thought to be pathognomonic of muscular dystrophy, but it is displayed in all cases where the muscles of the lower trunk and legs are weak If the child is laid on his back and told to get up he first rolls over on to his stomach Then he gets into a crawling position and slowly walks his hands backward toward his toes at the same time trying to straighten his knees and hips The latter, however, he cannot do without the help given by his arms as he walks his hands up his legs (Fig 224)

The shoulder girdles and upper arms are mostly affected long before the forearms though after the legs This leads to winging of the scapulæ and an inability to raise the arms above the head Children whose shoulder girdles are weak are described as slipping through the hands of anyone trying to lift them by holding them under the arms The flexors and extensors of the neck are often preserved to the end

The face is commonly spared in this form of dystrophy but the eyes in many cases appear to be unduly prominent or to show more of the sclerotics than usual giving a fleeting impression of hyperthyroidism During sleep

FIG 219 Two English brothers aged eight and six with pseudohypertrophic muscular dystrophy An elder brother is more seriously affected Note the winging of the scapulæ the weakness of the shoulder girdles preventing the arms from being raised further the low lumbar lordosis and the pseudo hypertrophy of the calves and thighs

the eyes often remain open the gruesome look of the face being most distressing to the mother

The involvement of the muscles of respiration is generally late but when it occurs is extremely serious since the inability to cough turns a trivial bronchitis to a rapidly fatal pneumonia

Swallowing is never or very rarely impeded and the sphincters of the bowel and bladder always remain normal

The nervous system is never affected but the tendon reflexes are often lost early in the disease from the loss of tone in the muscles

Irony is better shown in pseudohypertrophic dystrophy than in any other disease since while the legs grow weaker their muscles tend to grow larger This pseudohypertrophy often causes mothers to take a pathetic pride in their son s fine calves and is also the reason why they realize so late that the legs are weak Though the calves are most frequently almost

constantly affected any other muscles may enlarge. The extensors of the knees commonly undergo pseudohypertrophy at some stage, though the enlargement is often localized to one part of the quadriceps or may be so circumscribed that it is only obvious when the muscle contracts causing a sudden ball of muscle to rise up. This "ball" enlargement is also often seen in the muscles of the upper arm, the biceps, triceps and less often the deltoids, giving the impression of hard but minute bunched up muscles rather as if a pygmy boxer had given his muscles to a giant. Where the whole muscle is enlarged, as in the calves, the feel is typical. It has been described as woody or rubbery, but it is more like handling a piece of pickled pork. The lumbar muscles may be enlarged along their whole length standing out each side of the spine. On the other hand any group of muscles may waste from the beginning of the disease, especially in girls (Fig. 220). In general however the muscles of the calves and thighs enlarge and the muscles of the upper arms and shoulder girdles waste.

Fig. 220. English girl aged thirteen with muscular dystrophy. Note the low lumbar lordosis and wasting of calves and thighs without any pseudohypertrophy. Sexual development is, if anything, precocious.

Contractures. The importance of contractures in muscular dystrophy cannot be over emphasized. Indeed they often appear to be as much a symptom of the disease as the weakness of the muscles themselves. They are generally stated to occur late in the disease when the child has become too weak to move, so that by sitting all day in a chair his thighs and knees and feet become largely fixed in one position. This however appears too simple an explanation. It is quite common to see children who have had their Achilles tendon lengthened because it was too short months or even years before there was any suspicion that they were dystrophic and years before they ceased walking. On the other hand an occasional child is seen with no trace of any contractures who has been confined to his chair for months with no massage or treatment of any kind. One is indeed forced to wonder whether the inconstant but often dominant contractures are due not to the primary muscular weakness but to a primary change in connective tissue. Why this change is not found in all cases is obscure, but an analogy is seen in the rare primary involvement of the osseous system described below. The clinical relation of vitamin E to collagen is discussed further on p. 660.

Probably so little notice has been taken of contractures because the muscular weakness has been held to explain all the child's disabilities. Actually, however, the contractures are a major cause of his disabilities especially through their effect on the feet and hips.

The feet, as has already been mentioned, may be drawn down by the contractures of the Achilles tendons, so that the child in the early stages tends to stand or walk on tip toe, wearing out the toes of his shoes. As the deformity gets worse it is a very real hindrance to walking, even where the muscles of the legs are still moderately strong.

Contractures of the hips are seldom recognized yet it is they as much or more than the weak pelvic muscles which lead to the grotesque lumbar lordosis of these children To show that this is so it is only necessary to lie the child flat on his back, when he will be unable to keep his lumbar spine on the floor without flexing his thighs If he is laid on his face flexing his knees will cause his buttocks to rise in the air because the shortened muscles from his pelvis to his legs are thus stretched and so flex his body on his thighs Kneeling in an upright position is also impossible for many children who can still stand because in this position the shortened anterior thigh muscles pull the body out of the vertical as they are stretched by the flexed knees

Contractures of the knees are seldom an early complication of muscular dystrophy, though of course when they occur they are an added difficulty

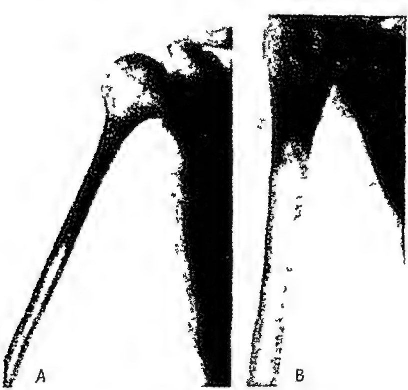


FIG 291 X ray A is of an Italian boy in America aged sixteen with muscular dystrophy and external concentric osseous atrophy (see text below) X ray B is of a normal boy of the same age

in walking Some limitation of movement at the elbows may occur early in the disease but it causes little disability

Osseous Atrophy For many years it has been recognized that in a very few cases a primary osseous atrophy may occur at the same time as the primary muscular atrophy Bramwell [333] believed that both these atrophies were manifestations of a change in the endocrine or sympathetic nervous system and Maybarduck and Levine [334] also hold both conditions are independent processes of the same disease The latter authors and Ashby and others [335] have each reported one case of their own and very fully reviewed the literature

The bone atrophy has been described as an external concentric progressive atrophy

The shafts of the long bones become progressively thinner, though the thickness of the cortex is not greatly reduced Apparently as the outside of the cortex is removed the inside is reinforced with further bone so that the slimness of the shaft chiefly occurs at the expense of the medullary

cavity. The length of the bones remains normal Cases are reported where the femora were no thicker than a little finger. The epiphyses of the bones are not decreased in size, so that the thin shaft connects two normally grown ends, giving a dumb bell effect. The ends, however, may show considerable rarefaction. The flat bones of the pelvis and scapula may undergo some rarefaction as well as the bones of the feet and patella Whether the small size of the winged shoulder blades seen in some cases is due to this atrophy or is secondary to the muscular weakness is uncertain. The fingers are thin and tapering

Rarely the mandible is involved, developing a short vertical ramus and an obtuse angle, so that only the molars can be made to meet.

It appears definite that these bony changes are one of the primary results of the disease and are not merely a secondary disuse atrophy brought about through the weak muscles For no bone changes may be seen in very advanced cases, and when they do occur they may be present where the muscular power is still unimpaired It is also significant that a case is recorded where there was both osseous atrophy and hypertrophy at the same time Further, a boy with muscular dystrophy and osseous atrophy had a sister whose muscles were normal but whose bones showed the same typical atrophy as her brother's Indeed it appears as if lack of vitamin E may sometimes only cause osseous atrophy, sparing the muscles completely. It is interesting to recall that vitamin E deficient rats may show osseous atrophy (p 623).

Other Symptoms. Pituitary dysfunction is said by Bramwell [333], and hypothyroidism by Wechsler [337], to be a common complication of muscular dystrophy, as indeed it may be in vitamin E deficient rats. However, sexual development in boys often appears to be precocious, a point noticed both by Bicknell and by Armstrong [336] Minot and Dodd [163] report that the urine of dystrophic boys contains œstrogens Most children with muscular dystrophy have unusually good teeth. Mental precocity is common in muscular dystrophy, since the children are too much in the company of adults, added to this precocity is often a lively intelligence which never suffers any change

Diagnosis When the disease is fully established it is unlikely to be confused with any other The diagnosis, however, is difficult in very young children who have been late in walking and then not walked properly, or in children whose early symptoms all appear to be merely the result of short Achilles tendons In such cases the estimation of the creatine and creatinine of the urine is of value, since the former is increased in muscular dystrophy and the latter diminished Creatine tolerance is also lowered These changes are found in all conditions where muscular function is impaired, so that they are really only of value in confirming that the child's symptoms are due to muscular weakness, where this is uncertain clinically. In children up to about the age of seven some creatine is normally present in the urine, but after this age it should disappear

A biopsy on the most affected muscles will confirm a doubtful diagnosis (p 614) The simpler electrical reactions of the muscles are of no interest, remaining normal as long as any contractile muscle is left

Course. Without treatment the child steadily gets weaker, generally dying from a respiratory infection somewhere in his teens Even with treatment if the chest expansion is bad when the child is first seen the outlook is very uncertain, as the most trivial bronchitis may end fatally in a few hours This is due to the child being so weak that he cannot cough up any mucus, with the result that bronchial obstruction and pulmonary collapse may rapidly occur The expansion of one side of the chest may also be hindered by the extreme scoliosis which develops in children left all day slumped in a chair

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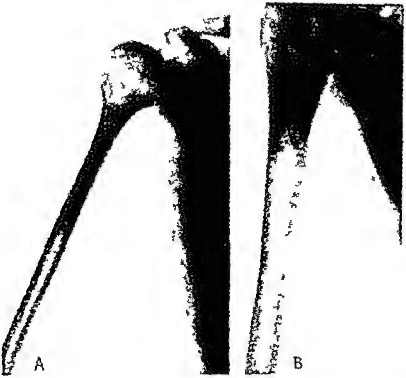

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lack of exercise, since they grow thinner with treatment even before they begin to move more freely. Most very advanced cases become extremely thin

Facio-Scapulo-Humeral Type of Landouzy and Dejerine [330]. This form of muscular dystrophy is sometimes referred to as *progressive muscular dystrophy* Intermediate forms, however, between it and the pseudo hypertrophic type are common, and the same changes in the muscle fibres occur in both. It seems, therefore, that the two diseases are fundamentally the same, though they differ in certain aspects

Thus in the facio-scapulo-humeral type both sexes are equally involved

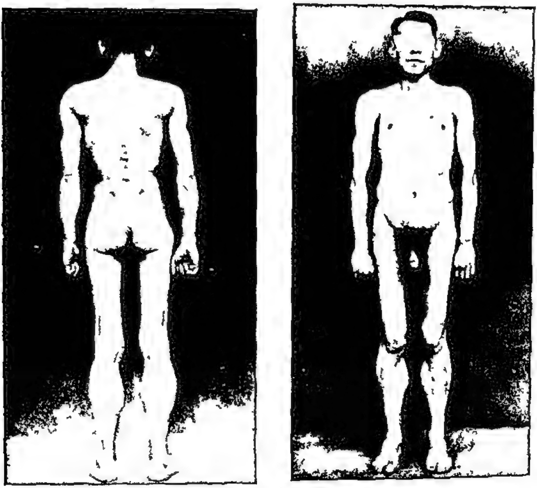


FIG 222 English man, aged twenty three, with pseudohypertrophic muscular dystrophy dating from early life, and progressing with unusual slowness Note the pseudohypertrophy of the calves and possibly the triceps, the wasted thighs and buttocks and the low lumbar lordosis Sexually he is very well developed

and there is a strong hereditary tendency. The age of onset is also different, being commonly in later childhood, though infants and young adults may develop the disease Progress is often so slow that the expectation of life is hardly diminished. One of the first cases to be described developed symptoms when he was twenty and died at sixty still pushing a hawker's barrow

As the name implies it is the muscles of the face, shoulders and upper arms which are chiefly affected. The facial muscles first become weak causing the myopathic facies, with its prominent lips, transverse smile and inability to close the eyes or frown. The expression has been called stupid and sanctimonious [330] With the involvement of the shoulder girdles the scapulæ become winged, and as the weakness spreads to the upper arms

many movements become impossible Lastly, the thigh muscles become affected Pseudohypertrophy is rare, and tends to be of the "ball" variety (p 639)

The Scapulo-Humeral or Juvenile Type of Erb [331] This variety tends to occur at adolescence or in early adult life and often progresses slowly. The upper arms and shoulder girdles are affected first and many years may pass before the legs become weak though progress is sometimes rapid

Treatment of All Forms of Muscular Dystrophy Bicknell [9] in 1940 first treated muscular dystrophy with vitamin E using whole wheat germ He claimed better results than were justified owing to his failure to realize

Fig 223 English woman aged forty with the facio scapulo humeral type of muscular dystrophy There is some difficulty not shown in the photograph in closing the right eye and showing the teeth Note the winged scapulae and weakness of the shoulder girdles preventing the arms from being fully raised

that encouragement alone can greatly increase the apparent muscular power of a child who has hitherto been left unstimulated and neglected in his bed or chair But over the last twelve years treatment in essence still being based on whole wheat germ a few, a very few, children have improved in a dramatic manner, a few have improved and have then remained stationary from before puberty to the twenties even marrying and having children, a few have remained stationary for years, many have continued to deteriorate Of those who have not deteriorated most have had as a back ground excellent and fresh food and no complicating diseases or chronic sepsis One observation to which it is easy to attach too much importance is that bread made at home from stone ground flour has been eaten by the majority though not all of the children who have not got worse Synthetic

racemic alpha tocopheryl acetate in doses ranging from 20 mg to 200 mg daily has been valueless

By chance owing to the circumstances of the patients only those children who have not responded to treatment with wheat germ alone have had their creatine and creatinine excretions measured But arrest or improvement on wheat germ is at best so slow and fluctuations in excretion from day to day and even from week to week are so great in all cases however treated [328] that significant alterations in excretion could only be demonstrated were they estimated almost continuously for many months Therefore until a rapid cure is discovered such investigations are of less value than the clinical progress of the child

Stone [345] in America has reported twenty five children treated with fresh wheat germ oil daily All improved and one completely recovered Progress was hastened by adding the vitamin B complex to the oil thus giving a mixture very similar to wheat germ and also ascorbic acid Minot and Dodd [163] giving large amounts of vitamin E improved two of five cases Donovan [254] treated a boy with dystrophy with three quarters of an ounce of wheat germ four times a day for six weeks with very marked success

No improvement however has been reported by the following investigators Harris [338] observed three dystrophic children for about nine months while they were taking up to 200 mg of alpha tocopherol daily by mouth He found no change in the excretion of creatine and creatinine or in their physical condition Minot and Frank [85] also failed to reduce the creatinuria in eight boys five to nineteen years old with varying doses of synthetic vitamin E or wheat germ oil In these boys the level of vitamin E in the blood (p 602) was normal before treatment and rose after Shelden and others [339] report eight cases of muscular dystrophy aged 5 19 28 28 30 34 38 58 Treatment was with either 100 mg of alpha tocopherol injected twice weekly or 50 mg given daily by mouth In every case 3 tablespoonfuls of wheat germ oil were taken after each meal The duration of the treatment is vague Ferrebee and his collaborators [340] treated twenty cases of whom thirteen were aged fifteen or less Treatment was continual for from four to fifteen months It consisted of the daily con sumption by mouth of two tablespoonfuls of wheat germ 110 to 190 mg of alpha tocopherol and 10 to 30 mg of vitamin B_6 Injections of 100 to 400 mg of alpha tocopherol were also given each week Pohl and Baethke [341] gave a wide range of oral doses of all forms of the natural and synthetic vitamin to fifteen cases for nearly two years with negative results In Spain Alemany Soler [343] and Allue Horna [344] have reviewed the results of many workers the great majority of whom failed to obtain any benefits from vitamin E though a few reported cures

Two criticisms of all these negative results can be made Firstly adult cases in whom the disease has lasted for many years cannot be expected to improve as the greater part of their weakness must be due not to muscle fibres which are still degenerating but to fibres which have completely disappeared

Secondly many of the young cases whose muscles should have been capable of some regeneration had large in most cases huge doses of alpha tocopherol In fact most of them were taking as much vitamin E as would be contained in a daily consumption of 50 lb of wholemeal bread Such quantities would appear to be far beyond any physiological requirements and to be thirty to forty times as large as those given to those cases which have been arrested or improved Nothing is known of where or how excess vitamin E is destroyed but since it is mostly stored in the muscles it well may be that they not only utilize vitamin E but also destroy any excess If this is so muscular metabolism might be overburdened by the effort of destroying large amounts The stimulating effect of large quantities of

vitamin E on phosphorus metabolism in muscle (p 595) may also be over whelming Indeed some of the American cases give the impression that they deteriorated faster when given massive doses of vitamin E

Along such lines as these probably lies the explanation of why small amounts of wheat germ or wheat germ oil and the vitamin B complex may cause a slow and continuous improvement in cases of muscular dystrophy while at best no lasting effect is gained by large amounts of alpha tocopherol insufficiently balanced by the vitamin B complex and the other constituents of wheat germ

From the above discussion it appears that the most satisfactory way of administering vitamin E *is to give whole wheat germ and fresh foods rich in vitamin E* About 1 ounce a day of wheat germ is ample Most children prefer it taken like a breakfast food before breakfast mixed with cold milk It is essential that the wheat germ should not be stale or rancid

Wheat germ oil and the vitamin B complex may be given to those unusual cases who dislike wheat germ Apart from the greater expense of this form of treatment there is always the uncertainty as to how fresh is the wheat germ oil and so how much of its vitamin E potency is retained In England wheat germ oil and wheat germ oil concentrates are usually sold in capsule form and are reported not to deteriorate The uncon centrated oil is probably best About 6 mgs daily of alpha tocopherol in this form should be sufficient

Wheat germ wheat germ oil and synthetic vitamin E all may cause an irritating urticaria

Rancid fat destroys vitamin E so that any food like rancid butter sour milk stale meat or cheese must be avoided (p 615)

The diet should also contain as much food rich in vitamin E as possible especially stone ground bread and green vegetables in large amounts The former is well worth the trivial trouble of making it at home Such a diet unsupplemented with any preparation of vitamin E is reported to have cured two children of muscular dystrophy and to have improved three others Bile salts should be given by mouth with the vitamin if there is any intestinal history suggesting that bile may be deficient since it is necessary for proper absorption (p 600)

Liquid paraffin or paraffin emulsions should not be used as aperients as they probably hinder the absorption of vitamin E (p 600) Constipation may be a severe trial to many patients and their families no single aperient suits every child so each type must be tried in turn senna pods or salines often being found the best

Sepsis delays or stops progress even if it is mild such as carious teeth sinusitis otitis media or ingrowing toe nails It cannot be overemphasized that sepsis must be treated

Infectious diseases such as scarlet fever tend to make the child worse for a time but on the other hand they may have no effect Children should be got up as soon as possible after illnesses Treatment with the sulphonamides and penicillin has no adverse effect on the muscular condition and has even been reported to be beneficial Since the mildest bronchitis may be fatal within a few hours (p 638) it should be immediately treated as if it were severe pneumonia

Treatment of Contractures Massage and Exercise Contractures in the majority of cases greatly increase the disability (p 639) Almost constantly there is a shortening of the Achilles tendon which added to the usual extreme weakness of the flexors and evertors of the feet makes walking difficult At the same time weak external rotators of the thighs may allow the feet to turn in so that the child trips up over them

Adjustable light splints worn at night will help to prevent further shortening of the calf muscles and also will tend to passively lengthen them Where splints are tolerated badly and the child sleeps on his back pressure

can be kept on the outer front of the sole by arranging that the feet press on a padded board pushed upward from the end of the bed Raising the head of the bed slightly can also be used for pushing the feet against their support

Very simple exercises should be given several times a day to make the child use the dorsiflexors and evertors of the feet and the rotators of the thighs It is remarkable how a child who has stopped using a muscle will not begin to use it again as it grows stronger, unless he is taught to do so

The contractures of the thighs and knees are partly checked if the child sleeps flat on his back, but it is not worth sacrificing sleep in trying to make children stay in a position they dislike For at least an hour a day all children, especially those with contractures, should lie flat on their backs on the floor or table with only a small cushion under their heads If the adductors of the thighs are so weak that the knees flop apart the legs should be tied loosely together with a scarf This rest is good for all children, and also tends to pull out the flexion contractures of the thighs and knees

When sitting the feet should be drawn under the child to increase dorsiflexion, but sitting is always a very bad position because it favours contractures of the thighs and knees Children instead should, as far as possible, recline so that the angle between their body and thighs is increased When children are too weak to change their own position it should frequently be changed for them Lateral curvature of the back must be avoided

Gentle passive stretching of the contractures should be carried out several times a day Laying the child on his back on a table so that his thighs hang unsupported over the end is an excellent method of stretching contractures of the thighs

Boots with high heels and the outer side wedged may be used to help in walking The boots must be light with flexible soles and as the calf muscles stretch, the heels must be cut down Spinal jackets and other forms of support must never be employed

Surgical treatment of contractures is seldom necessary, being generally corrected by the above methods In any case the longer an operation is postponed the stronger will be the muscles and the less the likelihood of a recurrence

General light massage is valuable for children who can move but little The great value of a masseuse, however, is to teach the child to use his muscles again as they grow stronger Very simple, varied, and amusing exercises must be given which the child and his mother can do several times a day Ideally every joint and muscle should be fully used every day Faradism should not be employed

Exercise is good as long as it is not forced Children will not over tire themselves when playing alone, and such games as "Red Indians" make them use combinations of muscles which are difficult with set exercises A puppy is an excellent playfellow for a dystrophic child Swimming or playing in the water is often possible when walking is not because the body is supported by the water On the other hand it is so important to avoid infections that public baths should never be used If the child gets cold he will for a few hours be considerably weaker

Glycine in the Treatment of Muscular Dystrophy Armstrong [336] has given a thorough review of the use of glycine in the treatment of muscular dystrophy, and has reported its effects in eighteen cases of his own many of whom were treated for more than three years He also followed the effects of treatment with biopsies on muscles It appears that glycine may give a temporary improvement in some cases but it is certainly of no lasting value in males and probably does not influence the ultimate course of the disease in females The experimental work of Ni which Armstrong mentions as supporting the use of glycine in dystrophy, is discussed on p 616 where it will be seen that Ni himself now does not consider that his experiments showed that glycine itself influenced the nutritional dystrophy of animals

The transitory clinical benefit of glycine is probably due to its increasing the power of normal muscle [342]

Other Diseases of Muscle *Amyotonia congenita* according to Stone [253 345] is the result of a very severe intra uterine deficiency of vitamin E Treatment with 2 to 4 ml of fresh wheat germ oil and the vitamin B complex gave excellent results Bicknell [9] also reported improvement in one case treated with whole wheat germ

Development and Growth Stone [253 345] treated a large group of children between the ages of one and five who were seen because of poor muscular development late standing and walking and inability to hold up their heads until they were two or three Treatment with the vitamin B complex had little effect but rapid improvement occurred when 8 to 12 minims daily of fresh wheat germ oil were given as well He believes that young children with muscular hypotonia are very mild cases of a vitamin E deficiency resulting from poor placental supplies

In eleven of seventeen premature infants Widenbauer [346] using wheat germ oil caused a rapid increase in weight after it had been stationary for some time His results were carefully controlled and appear important they are supported by one Spanish case [347] The malnourished children of Blackfan and Wolbach [326] appear to have had muscular degeneration due to lack of vitamin E

Scleroderma and Dermatomyositis Whole wheat germ has been reported as decreasing the creatinuria in three of five patients with this condition [348] and one case is said to have been cured by alpha tocopherol [349]

Fibrositis nad Rheumatic Diseases Steinberg [370] in 1949 stated that he had treated three hundred patients with generalized primary fibrositis a condition commonest in middle age where there is soreness in one or more groups of muscles often with bursitis which comes on after chilling or unusual muscular movements or stresses The twenty four hour creatine excretion is above 100 mg but otherwise all investigations give normal results and the patient is in good health The vast majority of such cases are cured by 300 mg daily of mixed tocopherols after which 50 to 150 mg daily are needed as a maintenance dose No controls are mentioned Steinberg states in an earlier paper that other forms of fibrositis do not respond but Ant and Cyan [371] claim again without controls that vitamin E is of value in all rheumatic diseases from rheumatoid arthritis and osteoarthritis to muscular rheumatism

VITAMIN E IN THE TREATMENT OF NEUROLOGICAL CONDITIONS

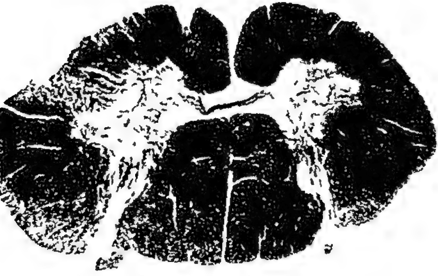
The experimental work of Einarson and Ringsted (p 618) raised high hopes that vitamin E would be of value in certain degenerations of the central nervous system—notably amyotrophic lateral sclerosis and tabes dorsalis The weight of evidence however is now against this though it must be admitted that the position is not yet clear From the clinical results discussed below it appears that alpha tocopherol used alone and in large amounts is at best of uncertain value in progressive muscular atrophy and amyotrophic lateral sclerosis But there are too many reports of vitamin E having had a transitory or prolonged effect on nervous degeneration for them to be completely ignored

The subject is extremely complex There is firstly the experimental
ack of vitamin E has initiated
n E to the diet will not check
Secondly the experimental
s shown that a degeneration
of the central nervous system in pigs can be produced by food deficiencies though the deficient factors have not been identified Thirdly synthetic vitamin E may be toxic in large amounts and may require the vitamin B

Fig 224. Typical movements of an English child with

complex for its proper utilization or destruction by the body Fourthly, as has been pointed out by Wechsler [351], similar nervous degenerations may, in different people, have different causes—thus some cases of amyotrophic lateral sclerosis may be due to a food deficiency, some to a vascular degeneration, and some to other causes Lastly there is Davison's outstanding work, to be discussed later, which strongly suggests that vitamin E profoundly modifies pathological changes in the central nervous system, even if it cannot prevent them

The impression left from the clinical reports is that we are still fumbling in the dark close to a solution of many of the nervous degenerations, but that until more light is thrown on the subject the patient's best chance is to be treated with natural vitamin E and the B complex, and a high intake of foods rich in the other vitamins This does not necessarily imply that the degenerations are caused by a food deficiency, but it does mean that any



Fig

deficiency which is hampering the nervous system in its recovery is removed The level of vitamin E in the blood is discussed on p 602

Amyotrophic Lateral Sclerosis and Progressive Muscular Atrophy Bicknell [9] in 1940 reported good results in two of four patients treated with whole wheat germ but later cases treated with either wheat germ or synthetic vitamin E have only shown at best a mild objective and subjective improvement for a few weeks before continuing to deteriorate In three cases and one reported from South America synthetic vitamin E has caused extremely painful cramps or spasms of the legs occurring chiefly at night Two patients regulated their own dose of vitamin E by these cramps which came and went as the dose was increased or diminished In one case the pain was so severe that a fatal collapse was feared though not more than 20 mg of alpha tocopherol was being taken A case of disseminated sclerosis also developed cramps whenever the daily intake rose about 9 mg The vitamin B complex appeared to increase the tolerance for vitamin E in these cases One patient who had marked euphoria gave up wheat germ oil because it changed his euphoria to depression

Wechsler [352] has reported sixty cases whom he treated with synthetic vitamin E in 50 mg doses daily either by mouth or injection He also

gave wheat germ oil the vitamin B complex foods rich in vitamin E and bile salts to aid the absorption of the latter Ten of his cases showed varying degrees of improvement two completely recovering The longest period of treatment was two years His paper [351] should be read for an excellent discussion on the general problems and implications raised by considering such cases are due to food deficiencies Some of his cases improved or got worse as the vitamin was given or withheld [351] He and others [53] report that in seventeen cases the average tocopherol content of the blood was 0 67 mg per 100 ml compared to 0 96 mg in twelve normal subjects the level in the latter varying between 0 59 and 1 62 mg Transitory clinical improvement with oral doses did not occur unless the level was raised by at least 0 4 mg this being only achieved by doses of at least 200 mg daily

Fig 226 From a non treated case showing extensive demyelination of the crossed pyramidal and left direct pyramidal tracts Compare with Fig 225 Myelin sheath stain

Injections of vitamin E caused a fall in the level in the blood which is analogous to the decrease in the level of vitamin A after it is injected

Einarson Ringsted and their collaborators [352] state that with 90 mg of alpha tocopherol their results from eight cases furnish some basis for believing that vitamin E may have an effect at least on neurogenic muscular atrophy

Meller [353] treated fourteen cases with 100 to 250 mg of alpha tocopherol daily by mouth or by injection for periods of three to seven months three recovered and seven improved Rosenberger [354] relieved some of the symptoms in eight of nine patients with a diet rich in vitamin E synthetic aneurine and 100 to 200 mg of alpha tocopherol given orally His patients were observed for about a year Donzallaz and Monnier [355] in Switzerland treated an interned Alsatian soldier for three months with 30 mg of alpha tocopherol daily by mouth There was a considerable degree of recovery writing and other activities becoming possible During a short period with no treatment his weakness returned Gotten [356] reports good results in

AMYOTROPHIC LATERAL SCLEROSIS

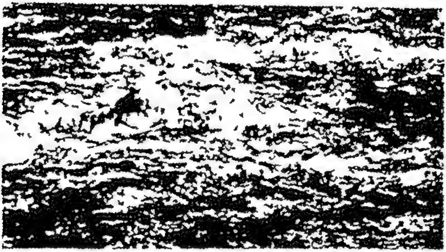


Fig 227 Case I Insular myelin sheath destruction from a case treated with vitamin E Compare with Fig 228 Myelin sheath stain × 240

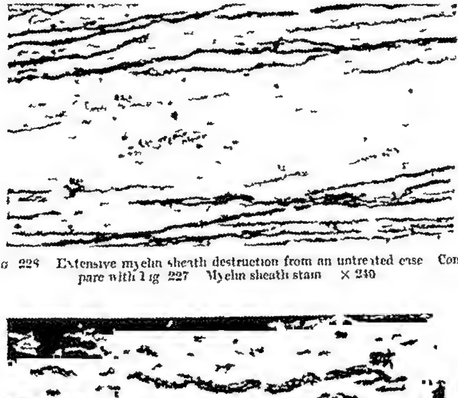


Fig 228 Extensive myelin sheath destruction from an untreated case Compare with Fig 227 Myelin sheath stain × 240

Fig 229 Ca ... res in parts of ... se of amyotrop ... with Figs 227

AMYOTROPHIC LATERAL SCLEROSIS

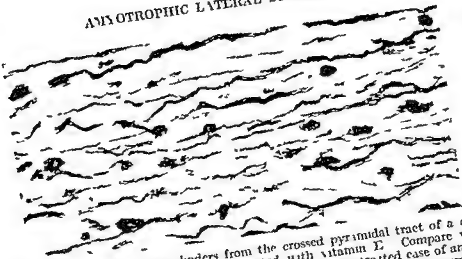


FIG 230 Case I Axis cylinders from the crossed pyramidal tract of a case of amyotrophic lateral sclerosis treated with vitamin E Compare with Fig 231 from a normal case and Fig 232 from an untreated case of amyotrophic lateral sclerosis In Fig 230 there is a slight diminution in number, swelling and slight tortuosity of axis cylinders when compared with the normal in Fig 231 and the severely diseased fragmented and swollen axis cylinders of the untreated case of amyotrophic lateral sclerosis in Fig 232 Bielschowsky stain × 480

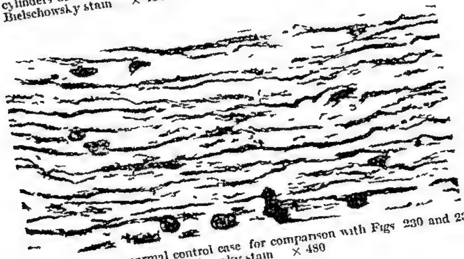


FIG 231 From a normal control case for comparison with Figs 230 and 232 Bielschowsky stain × 480

FIG 232 From an untreated case of amyotrophic lateral sclerosis For comparison with Figs 230 and 231 Bielschowsky stain × 480

two patients, Viets [352] improvement in one patient among twenty one, and Slaughter and Cleckley [357] one who completely recovered on 1 drachm of wheat germ oil daily.

Pakenham-Walsh in a personal communication states that of three cases treated with wheat germ one definitely improved and the others did so for a short time, and then continued to degenerate Gutiérrez-Mahoney [231]

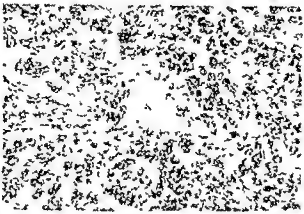


FIG 233 Case I Almost complete absence of fat in the pyramidal pathways from a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Fig 234 from an untreated case showing lipoid deposits throughout and in the perivascular spaces Sudan III stain × 100

cured six of nine cases Three relapsed when they ceased to take wheat germ oil concentrate, but again recovered when they resumed. One man, who could only walk with a stick, after two years' treatment ran well and returned to work

Weinberg [358] gave wheat germ oil to one man who had developed signs suggestive of amyotrophic lateral sclerosis while taking sulphathiazole The neurological condition was cured though the concentration of the sulpha-

FIG 234 From an untreated case of amyotrophic lateral sclerosis, for comparison with Fig 233 Sudan III stain × 100

thiazole in the blood remained unchanged Other vitamins failed to prevent the recurrence of neurological signs each time the drug was given This is reminiscent of the effect of sulphathiazole in accelerating the development of muscular dystrophy in vitamin E deficient animals (p 625), though not in man (p 645)

Davison [359], who has very kindly allowed us to reproduce his most important and unique *microphotographs* in Figs 225 to 238, treated ten cases—six men and four women—in the manner advised by Wechsler. After death histopathological examinations were made, forty untreated cases being

AMYOTROPHIC LATERAL SCLEROSIS

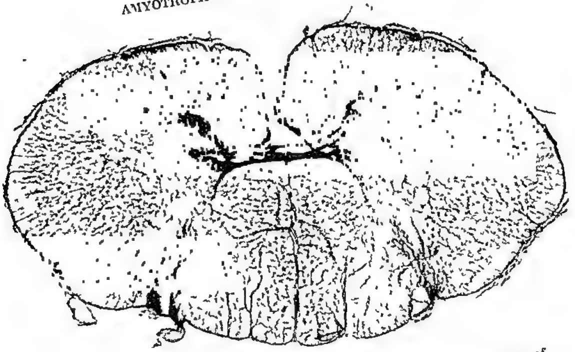


Fig. 235 Case I Lack of gliosis in the pyramidal tracts from a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Fig 236 from an untreated case of amyotrophic lateral sclerosis showing dense gliosis in the crossed pyramidal tracts Holzer stain

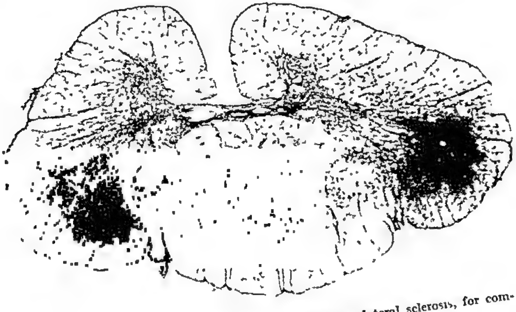


Fig 236. From an untreated case of amyotrophic lateral sclerosis, for comparison with Fig 235 Holzer stain.

used as controls In the six cases who were considered to have had adequ
treatment for a sufficient period of time—five weeks to over seven month
the degeneration of the cells of the bulbar nuclei and anterior horns was
affected But the other pathological changes were checked or rever
The demyelinization of the crossed pyramidal tracts was not one tentl

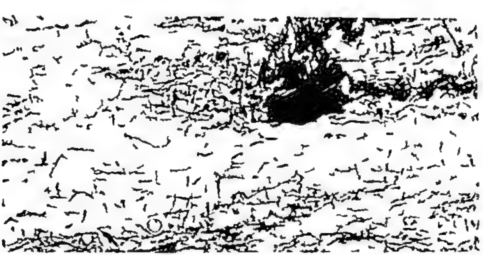


Fig 237 Case I Slight insular gliosis from a treated case of amyotropl c lateral sclerosis Compare with Fig 238 from an untreated case In Fig 238 the gliosis is dense Holzer stain × 200

that in the untreated cases and indeed was hardly visible to the naked
in stained preparations The changes in the axis cylinders were also far
pronounced The dense gliosis plainly visible in stained preparations f
the untreated cases was virtually absent and in most cases the fatty depo
were also greatly reduced This outstanding work suggests like that on

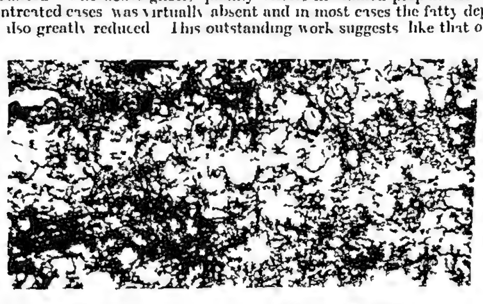


Fig 238 From an u treated case of amyotropl ic lateral sclerosis for comparison with Fig 237 Holzer stain × 200

muscular dystrophies that while vitamin E may play an essential part
treatment it is not in most cases the whole answer to the problem

Worster Drought and Shafar [360] have reported that synthetic vitam
E in 30 mg doses daily had no effect in twelve cases apart from one w
appeared slightly better They noted that mental depression was relieve
Doyle and Merritt [362] gave eight patients an excellent diet the vitamin
complex cod liver oil and 7 ml of wheat germ oil daily all by mouth a
synthetic vitamins E (60 mg daily) B_6 aneurine and liver extract l
injection The results were negative in both the late and early cases Sheld

and his colleagues [339] also failed to improve ten cases treated with 45 ml of wheat germ oil with each meal, supplemented daily by 50 mg of synthetic vitamin E by mouth or 100 mg twice weekly by injection A later report following more intensive therapy on further cases is equally negative [363]

Denker and Scheinin [361] have reported that eleven patients, including one vegetarian, did not respond to about 100 mg daily of synthetic vitamin E given by mouth or by injection Four further patients given 250 mg daily and 100 mg of vitamin B_6 did not improve There was no evidence, either from the histories, gastric analyses, or radiography, that intestinal disease [illegible] Klinsman and Franz [340] gave wheat [illegible]

were being taken daily

Goodhart, of the Montefiore Hospital of New York, tells us that twelve cases treated with synthetic vitamins E and aneurine showed no improvement De Jong [352] has only noted at best a fleeting subjective improvement in twenty six patients treated for upwards of a year with wheat germ oil, yeast, and injections of alpha-tocopherol up to 240 mg daily In the discussion follow [illegible] paper Moersch and Viets reported, without giving details of [illegible] one improved Furt [illegible] velve patients treated with wheat germ oil [illegible] nty-seven months

Disseminated Sclerosis and Other Conditions Meller [353] and Couperus [76] found that large amounts of alpha tocopherol were valueless for *disseminated sclerosis* but Dowd [365] believes that they may have a dramatic effect when combined with extensive vitamin and symptomatic therapy Stone [366, 367], using wheat germ oil for children and 50 to 150 mg daily of mixed tocopherols for adults, both in conjunction with other therapy, has reported excellent though uncontrolled results in *tabes dorsalis* and *congenital non obstructive hydrocephalus* and in *psychiatric disorders,* especially those with depression, anxiety and fatigue, benefit in the last of these has also been reported by Michael and Ruggles [368] Vitamin E is said to raise the level of cholesterol and fatty acids in the blood of schizophrenics [375] For *Sydenham's chorea*, between the ages of six and nineteen years, Dowd [369] gave every alternate patient 90 to 225 mg daily of natural alpha tocopherol In only two of the seventeen untreated cases was there improvement, while all the treated cases were symptom free in a month Subsequent treatment of the untreated cases abolished their symptoms Before treatment all the patients had had routine rest in bed and sedatives, but in almost all choreiform movements, a rapid pulse and joint pains had persisted

FURTHER CLINICAL USES OF VITAMIN E

Cardiac Diseases Vitamin E is valueless in cardiac diseases The subject evoked much interest in the later nineteen forties because the Shute brothers and others [376, 377] claimed that cardiac degenerations and, especially, angina of effort were greatly improved by massive doses of vitamin E But all their work is remarkably vague without any controls, and when, rarely, objective findings such as electrocardiographic changes are reported, these again are uncontrolled and indefinite

The Council on Pharmacy and Chemistry of the American Medical Association [378] early in 1950 repeated their earlier statement that "vitamin E is *of no value in coronary* heart disease, hypertension or rheumatic heart disease" This statement is borne out by the most carefully controlled work of many investigators [67, 378 386] Further, the level of vitamin E in the blood of patients with cardiac disease is normal (p 602) and the experimental reasons advanced for using vitamin E appear com-

pletely fallacious bits and pieces have been taken from various papers which when read in their entirety do not justify the use of vitamin E Again the cardiac condition in animals deprived of vitamin E (p 614) is *not analogous*

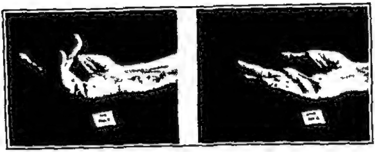
mixed tocopherols or alpha tocopherol confer benefit not by remedying a deficiency but by alleviating an anoxia then the simple answer is that *clinically* they do not

Peripheral Vascular Diseases etc Here vitamin E may be of value In intermittent claudication the only properly controlled work is that of Ratcliffe [387] in England who gave 400 mg of racemic alpha tocopheryl acetate daily for three months and judged the effects with a walking machine Of forty one patients with intermittent claudication who at most could only walk 500 yards thirty four improved to the extent of being able to walk at least 880 yards with little or no pain while of twenty five control patients only five improved to a similar degree Boyd and his *colleagues* [388] treated seventy two patients with intermittent claudication comparable to that of Ratcliffe s patients using the same dose of vitamin E Fifty nine improved—some walking a mile with no pain—and thirteen were not benefited In four very severely affected patients the nutrition of the feet improved though the pain was not relieved Treatment must be continued for three months before abandoning it as valueless The experimental evidence on arteritis is given on page 623 where it can be seen that vitamin E has only been shown to prevent perhaps arterial degeneration caused by grossly abnormal conditions

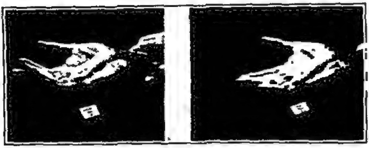
Ulcers of the Legs Since from the above reports vitamin E might be expected at least to improve some types of ulceration it is disappointing to find that reports claiming benefit [389] are vague and uncontrolled and have not been confirmed in a small number of cases [390]

Thrombosis, Capillary Permeability etc Kay *and others* [391] *state* that nineteen patients out of two hundred and thirty eight suffered from post operative thrombosis following major operations such as intestinal resection gastrectomy and pneumonectomy *But there were no thrombotic complica*tions in a similar group of one hundred and seventy five patients who were given post operatively 200 mg of alpha tocopherol every eight hours by mouth combined with intravenous injections every twenty four hours of 10 ml of 10 per cent calcium gluconate This report deserves to be considered seriously The experimental work described on p 623 suggests that vitamin E might well be of value in post traumatic thrombosis

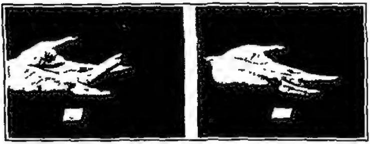
Capillary Permeability etc Minkowski [392] from very careful work which merits attention claims that giving 400 to 600 mg of alpha tocopherol during labour increases capillary resistance and decreases hæmorrhage in the newborn and premature infant This receives some slight support from the beneficial effect which 200 mg daily of vitamin E is said to have in dogs rendered purpuric by stilbœstrol [283] and from claims that five adults with thrombocytopenic purpura three women with menorrhagia and bruising and patients with a terminal purpura all had their purpura relieved within one or two weeks by daily doses of 200 mg to 400 mg of alpha tocopheryl acetate [283] Akin to this is the reputed good effect of vitamin E in sclerœdema in infants [393] which is also reminiscent of the œdema in vitamin E deficient animals (p 619) The work of Owens and Owens [73] on retrolental fibroplasia in premature infants again appears to be possibly related to vascular permeability Each alternate infant was given *thrice* daily 50 mg of a water miscible preparation of racemic *alpha tocopheryl* acetate Of eleven infants so treated not one developed retrolental fibroplasia while of fifteen untreated infants five were affected Of the next twelve infants given vitamin E only one was affected and he was so ill he



Case 1.



Case 2.



Case 3.

FIG. 239 Three hands with Dupuytren's contracture before treatment and after the patients were given 200 mg daily of alpha-tocopheryl acetate by mouth, for three to five months.

FIG. 240. Dupuytren's contracture before treatment, after treatment with alpha-tocopheryl acetate and later after surgical treatment.

only started treatment eleven days after birth Of sixty-three infants born before vitamin E therapy was introduced, twelve were affected The condition was even arrested—which is most unusual—in five of nine infants On the other hand Laupus and Bousquet [394] report that of sixty-five infants treated with the same dose of vitamin E, eleven were affected The incidence of retrolental fibroplasia varies greatly in different hospitals, possibly it has several causes and so vitamin E will only be of value in those hospitals which in some manner are giving their expectant mothers or the infants a faulty diet

Retinal degenerations in adults are said to benefit from vitamin E [395], as is syphilitic interstitial keratitis [396]

Dupuytren's Contracture, Penile Fibroses and the Collagenoses Russell Thomson [397] in Scotland has reported that 200 mg daily of racemic alpha tocopheryl acetate causes a slow *softening and stretching of the fibrous* bands of Dupuytren's contracture (Fig 239) He treated twelve cases, all with success, though four hands required operative treatment to complete the cure which generally started in six to eight weeks, though dramatic improvement even occurred in a fortnight in two further cases only treated with 30 mg

and acute

[153] who

only three did not benefit Treatment must be continued for more than three months before being abandoned as useless Negative [418] and positive [419] results have been reported by other workers, who gave adequate doses for several months Possibly the general dietetic background is the deciding factor in the response to vitamin E King [398] gave 300 mg of racemic *alpha tocopheryl acetate daily to thirteen patients and reported that six* abandoned the treatment because of nausea, giddiness, headaches, tinnitus, swelling of the tongue, etc Of the seven who continued for one or two months, only one may have been slightly better This paper is incomprehensible since vitamin E has been frequently given in doses far larger than these with *no* ill effects

Penile Fibroses Scardino and Scott [400] treated twenty-seven cases of Peyronie's disease with 300 mg daily of mixed tocopherols and obtained good results in thirteen, fair in nine and none in five Treatment in some cases lasted for eighteen months No relapses were observed a year after treatment was stopped. There were no control cases Scardino and Hudson [401] report that urethral strictures, whatever their aetiology and however long they have been present, often respond to mixed tocopherols in daily doses of 200 to 1,200 mg *Of twenty two cases response was good in* fifteen, fair in four and absent in three There were no controls and the duration of the treatment is vague

The Collagenoses Burgess [399] in a paper giving no clinical details states that daily doses of 100 to 600 mg of mixed tocopherols generally

en sclerosis et atrophicus, morphea and

of the latter condition were treated by

to 600 mg of racemic alpha tocopheryl

acetate after six to nine weeks, nine patients were cured, two probably cured, one better and one unaffected In *lupus erythematosus* Burgess and Pritchard [403] have reported excellent results twenty five cases were treated with very large doses of mixed tocopherols both orally and by mouth, and only one failed to improve Morgan [404] however, found vitamin E *useless for this condition* and Sweet [405] found it useless for all the collagenoses

Miscellaneous Conditions Diabetics have been said to benefit dramatically from vitamin E [406, 407] but careful work [408, 409, 410] shows this is not so The level of vitamin E in the blood of diabetics is normal (p 602) and there is no experimental evidence (p 596) suggesting that this form of

treatment is warranted In a few cases of neural leprosy, 20 mg daily of alpha tocopherol for two months, combined with sulphones, has given remarkable results [417]

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CHAPTER IX

ESSENTIAL UNSATURATED FATTY ACIDS

VITAMIN F

AND OTHER MINOR FAT SOLUBLE VITAMINS

Burr and Burr [1] in America in 1929 and 1930 first described the "fat deficiency disease" of weanling rats which occurs when the diet is deficient in certain polyunsaturated fatty acids, though for some years before this it was becoming increasingly plain that the body cannot satisfy all its fat requirements by synthesis from the carbohydrates of the food. Further work in America and in England—especially by Smedley-Maclean and her colleagues—confirmed and amplified the experimental work of Burr and Burr and showed that other species beside the rat require these polyunsaturated fatty acids. For man, however, their importance is still uncertain, though their deficiency is probably one of the causes of infantile eczema.

Chemistry The three essential unsaturated fatty acids are —

Linoleic acid—
$CH_3(CH_2)_3(CH_2 CH{:}CH)_2(CH_2)_7 COOH$

Linolenic acid—
$CH_3(CH_2 CH{:}CH)_3(CH_2)_7 COOH$

and Arachidonic acid—
$CH_3(CH_2)_3(CH_2 CH{:}CH)_4(CH_2)_3 COOH$

The first two of these are colourless oils while arachidonic acid forms colourless crystals with a melting point of 77° C. All three are soluble in organic solvents and alkalis but not in water. Their particular chemical properties depend on the positions of their double bonds. For details of their isolation from fats and their chemical reactions and estimation the reader should consult the books and papers by Smedley-Maclean [2, 3], Hilditch [4], Rosenberg [5] or Brooker and Shorland [6].

From the dietetic point of view the most salient property of the unsaturated fatty acids is the ease with which they become rancid through oxidation when exposed to the air. The first products of this oxidation, which does not in the early stages involve the double bonds [7], are labile peroxides which alter further into keto-hydroxylic derivatives and then either polymerize or undergo disruption with the formation of aldehydic compounds [4]. These products of oxidative rancidity are of the greatest importance since they are not only toxic in themselves [8], causing for instance anaemia [9], but also their presence in food leads to the oxidation and destruction of other substances, so that they may convert diets containing ample carotene and vitamin A (p 15) or ample vitamin E (p 615) or ample of the vitamin B complex [10, 11] into diets deficient in these vitamins.

Physiology *Synthesis* Linoleic and linolenic acids are found in large quantities in vegetable fats, but the presence of arachidonic acid is uncertain [4]. In fish oils [4, 12, 13, 14] it is doubtful whether any of the three essential unsaturated fatty acids occur in forms identical with those found in vegetable and animal fats unless, which is very rare, the fish have consumed large amounts in their diet [4]. Warm blooded animals have generally been

thought not to synthesize the polyunsaturated fatty acids but it may well be that they do, though in such small amounts that there is not enough for life during growth Thus Barki and others [15] fed adult rats on a restricted and also deficient diet until they had lost half their weight They were then allowed to eat as much as they would of the deficient diet They rapidly regained weight but in about two weeks developed deficiency symptoms These symptoms, however, ultimately vanished, though the diet still contained no essential unsaturated fatty acids With the spontaneous recovery of the rats there was an increase in the amounts of linoleic and linolenic acids in the body It appears, therefore, that the adult rat can synthesize enough of the essential acids to remain in good health, but only as long as no extra demands are made by growth Linoleic acid can be converted in the body to arachidonic acid [15, 16] and linolenic acid to hexaenoic acid [16] Widmer and Holman [16] also report that pentaenoic acid may be found in the kidneys and blood of deficient animals subsequently given linolenic acid, but they consider that this is probably due to abnormal synthesis by kidneys damaged by the deficiency (p 675) since the acid appeared in no other organ Holman and Taylor [17] are uncertain as to exactly what acids are formed from arachidonic acid Linoleic acid decreases stores of linolenic acid [15], possibly because the latter needs the former for its metabolism [19] In chicks, on the other hand, Reiser [18] in work which needs confirmation, states or implies that though linoleic and linolenic acids cannot be synthesized, yet once supplied they can change into each other or any polyunsaturated acid Vitamin E is said not to affect the formation of more unsaturated fatty acids from those less unsaturated, though benzoyl peroxide may [110]

Absorption No particular work has been done on the absorption of the essential unsaturated fatty acids from the gut, but there is no reason to suppose this differs from that of other fatty acids, except that in ruminants linolenic acid appears to be destroyed by the prolonged digestion in the rumen [6] Absorption from the skin is excellent [20, 21] In rats placental transfer appears to depend on the mother having ample reserves, since when these are low none are passed to the fœtus [22] In eggs from hens given linoleic acid it is said that there are arachidonic and pentaenoic acids but not hexaenoic acid [18]

Storage, Utilization and Destruction The polyunsaturated fatty acids are stored chiefly in the phospholipoids [… [16] arachidonic acid and haxaenoic acid are … ving percentages of the total fatty acids he … 10 4, kidney 4 2 and 1 3, brain 2 8 and 6 9, blood 3 2 and 3 1, muscle 1 9 and 3 3 intestine 1 8 and 1 9, skin 0 6 and 1 5, depot fat 0 1 and 1 7 In lecithin from bovine liver [24] linoleic acid forms forty five per cent of the unsaturated fatty acids and arachidonic acid only thirty one per cent , the latter acid forms 5 5 per cent of the total fatty acids of the suprarenals and four per cent of those of the spleen, while for the liver and thyroid of the pig the figures are 2 0 to 7 7 per cent and 0 4 per cent [25] Linoleic acid rather than arachidonic acid tends to be stored in the depot fat in man, for instance, linoleic acid forms 8 2 to 11 0 per cent of the total fatty acids while arachidonic acid forms only 0 3 to 1 0 per cent [23] During depletion linolenic acid increases as the other acids decrease [66] When the polyunsaturated fatty acids are given to a depleted animal stores are built up again in the following order, first in the heart and then in the liver, brain, kidney, blood and skeletal muscle [16] In the horse and rabbit [6] and possibly [26], but not certainly [6] in the pig large amounts of linolenic acid are present in the depot fat, and also in that of the rat [26] when this animal consumes large amounts of the acid , but ruminants store none [6]

The essential fatty acids are most carefully husbanded in the body Thus Smedley Maclean and Hume [27] for instance, report that after nearly six months on a deficient diet, when all growth has ceased (p 674), the skin

and subcutaneous tissue contains traces of the polyunsaturated acids while the total content of the carcase fat is still more than half of what it was at weaning. Even after a further six months on the deficient diet there is little further depletion and the liver contains about 0.3 per cent of these acids, probably as dihydroarachidonic acid [28]. In complete starvation arachidonic acid is spared until about seventy five per cent of the fat reserves have been used up; then there is a rapid fall as the rat becomes seriously ill [29].

Little is known about what activities of the body lead to depletion of its stores of the essential unsaturated fatty acids, though probably growth is the most important. Thus when deficient rats are given arachidonic acid so that growth is resumed, a large proportion of the acid cannot be recovered from the animals [13, 17], and in mature rats deficiency symptoms only occur during rapid gain of weight (p. 672). In symptomless chronically deficient mice a slight loss of blood or X ray trauma will precipitate deficiency symptoms [37]. Rapidly growing tumours decrease storage—though they grow normally even when storage is very low [30]. During lactation there is some loss through secretion in the milk. The various physiological and pathological conditions which have been stated to alter the amount of unsaturated fatty acids in the serum have been reviewed by Hansen (p. 677).

Activity of Fatty Acids. "Fat deficiency disease" is an unfortunate name since the disease is not due to a deficiency of fat in general, but to a deficiency of the essential unsaturated fatty acids. The disease can neither be prevented nor cured by saturated fatty acids [31, 34], by oleic acid [32], by isomeric oleic acid [32] with the double bond at 12, 13, by alpha elæostearic acid [33], by erucic acid [32], by chaulmoogric acid [32, 34], by ricinoleic acid [32, 34], or by some of the oxidation products of linoleic and linolenic acids [34].

Cod liver oil [13] se
no effect, though one of
growth [34]. Cod liver
and hexaenoic acids in

Prevention and cure of the fat deficiency disease is brought about by linoleic acid, linolenic acid, some of the oxidation products of these [34], and arachidonic acid. The activity of the first two of these appears to depend on having double bonds at 9, 10 and 12, 13, but not on their carboxyl group, since this may be changed for an alcohol group [32]. Arachidonic acid is about three times as potent as linoleic acid for promoting growth [32] and linoleic acid is about six times as potent as linolenic acid [19, 34]. For

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ibined with the fact that docosahexaenoic acid from cod liver oil promotes growth but has little or no effect on the skin [34], has led Hume and others [34] to the conclusion that the essential fatty acids have not identical actions. Greenberg and his co-workers [19], however, state that linolenic acid when given with linoleic acid is as efficient as the latter for promoting growth and the cure of the skin symptoms: linoleic acid sparks linolenic acid. Widmer and Holman [16] agree with Hume believing that the "fat deficiency disease" is really two diseases, one due to a deficiency of linoleic or arachidonic acid and the other due to a deficiency of linolenic or hexaenoic acid.

The optimum daily intake of linoleic acid for weanling rats is for females 10 to 20 mg. and for males probably 100 mg. or less [19, 38], arachidonic acid being about three times as effective [32]. The needs of some bacteria for unsaturated fatty acids can be met by linoleic or linolenic acids [54].

Symptoms of the Fat Deficiency Disease. Weanling rats have chiefly been

used for studying the effects of a deficiency of the essential unsaturated fatty acids, though isolated reports suggest that the mouse [37, 39] and the dog [40, 64] respond to a deficiency in the same way as the rat. The chick responds in a different manner, discussed at the end of this section. Observations on man are described on p. 676.

The symptoms and signs of the fat deficiency disease in weanling animals are retarded and ultimately arrested growth—accompanied by a raised metabolic rate—altered fat and water metabolism, changes in the skin and hair, renal degeneration and impairment of the sexual functions. Adult rats [15] and adult mice [37] develop no obvious symptoms unless under the strain of growth [15] or trauma [37].

Growth. During the first three or four months of the deficient diet growth continues though at a diminished rate; after this the weight remains stationary or declines slightly though the animals may survive for many months [2, 27, 32, 34]. The consumption of food, however, remains as high as that of animals on a normal diet [2] so that the basal metabolic rate is considerably increased [41].

Fat and Water Metabolism. Burr and Burr [2] originally believed that deficient animals could not synthesize fat, but it is now known that fat metabolism remains virtually normal. Fat is synthesized from carbohydrate since after a carbohydrate meal the respiratory quotient rises above unity [41] and this strong evidence for the synthesis of fat is confirmed by Smedley-Maclean and others [27] finding that the proportion of fat in the subcutaneous tissues and, to a lesser extent, in the carcase is actually higher in deficient than normal animals while in the liver it remains normal [27] or raised [28, 37]. That fat is burnt normally is shown by the low respiratory quotient of fasting deficient rats [50]. The rate of synthesis [42, 43] and breakdown [42] of phospholipoids remains normal in the liver [42, 43] and kidney [42] of the deficient animal, while it is increased by about one third in the muscles [42]. On a high fat diet fat storage is impaired but less in females than in males [55].

The sparing action of fat on aneurine (p. 197) is not affected by the degree of unsaturation of the fat [44].

The lipotropic effect of choline depends, according to Engel [45], on the presence of linoleic acid. But this is probably a secondary effect due to the acid permitting growth and so fat consumption [30].

About twice the normal amount of water is drunk by the deficient animals although there is no increase in the amount of urine [2], and this may be the only symptom in adult mice [37].

Skin and Hair. Both Burr and Burr [2] and Hume and her collaborators [34] report that dryness and scurfiness of the fore and hind paws is the earliest and most constant symptom of the deficiency, occurring while the animals are still growing, after only ten days to a few weeks on the deficient diet. The condition of the paws returns to normal within three to five weeks when linoleic or arachidonic acid are given. The skin over the rest of the body also becomes dry and scurfy and the hair thin, especially over the face and round the eyes [32]. The tail may be hairless and corrugated or annulated or even necrotic but these changes are inconstant and irregular and heal very slowly [34]. Some observers [32] even find all skin changes are slight and unreliable indicators of the deficiency—a finding possibly due to the climate of different laboratories, since the type of weather which causes chapping in man accentuates the skin changes in the deficient rats [41, 46]. Vitamin B_6 has been thought to have some relationship to the essential unsaturated fatty acids since its deficiency also causes somewhat similar changes in the skin of rats, but the only relationship is the obvious one that [illegible] changes than a deficiency of only [illegible] nin B_6 does not enable

Williamson [49] reports that the epidermis of the fat deficient rat becomes thicker and more differentiated the *stratum granulosum* being especially distinct and the horny layer thick Cells showing mitosis may be four or even eight times as numerous in rats kept on the deficient diet for nine weeks as in normal rats [3]

Renal Degeneration Hæmaturia was a frequent finding in the rats originally described by Burr and Burr [2] indeed these workers have since reported renal lesions in all their animals [46] Other workers however have frequently failed to find any hæmaturia in rats [32 34] and no renal lesions in dogs [40] The lesion in the kidney is said to be calcification in the cells of some renal tubules and necrotic areas in the renal medulla [51] Burr and Burr [2] consider the renal lesion which is made worse by a high protein diet is the cause of death

Sexual Functions Ovulation though rarely it may remain normal [32]
is absent
ted fatty
Mating
may take place if ovulation occurs but gestation is prolonged and ends in resorption in one fifth of the animals [52] or in protracted labour with excessive hæmorrhage [36] The litters are undersized and often so weak that they soon die [8 52] In mice no litters are born and it is even doubtful if conception occurs [37] Lactation is possible if the mothers are given the missing fatty acids but is poor unless other fat is also added in reasonable amounts to the diet [52]

Males show a loss of the normal sexual responses and will seldom mate when they do they are sterile [2] This sterility can be cured [53] After nine months on the deficient diet most of the tubules of the testis are lined with spermatogonia and one or more layers of spermatocytes no maturer cells being present Considerable numbers of tubules have no epithelium and multinucleated giant cells may be found in the lumen [53]

Chicks cease to grow and become so intensely œdematous that they may appear almost transparent [18] The relation between the unsaturated fatty acids and vitamin E in the nutrition of the chick is discussed on p 619

Distribution in Foods and the Effects of Storage and Hydrogenation Vegetable and seed fats though not margarine may contain large amounts of linoleic and linolenic acid but for all practical purposes contain no arachidonic acid The highly unsaturated acids of fish oils appear not to contain the essential fatty acids in the forms found in land plants and animals cod liver oil and other fish oils having little effect on the cure of the fat deficiency disease (p 671) though one of the highly unsaturated acids of cod liver oil does bring about a resumption of growth (p 674) Animal fats according to the diets of the animals may contain large amounts of the essential unsaturated fatty acids (p 672) and human milk [56] in contrast to cow s milk is an excellent source providing as well higher unsaturated fatty acids

The ease with which the essential unsaturated fatty acids are destroyed by oxidative rancidity in the presence of air (p 671) means not only that they themselves may be destroyed when food is not fresh but also that their rancidity may destroy other essential constituents of the diet (p 671) besides the vitamins (p 671) The hydrogenation of the vegetable fats of margarine while it has the advantage of enabling manufacturers to sell a product which delights shopkeepers by remaining tasteless for many months in a warm room has of course the drawback that much of the essential unsaturated fatty acid is converted to saturated fatty acid

The following figures have been largely taken from *The Chemical Constitution of the Natural Fats* by Hilditch [4] to which the reader should refer for further information

Food	Per Cent of Essential Unsaturated Fatty Acid
Animal Products (p. 672):	
Butter	1·9–4·0
Beef Fat	1·1–5·0
Lard	5·0–11·1
Mutton Fat	3·0–5·0
Liver Fat	3·0–7·0
Milk (Cow)	0·15–0·23
(Ewe)	0·36
(Goat)	0·22
(Human)	0·39–0·40
(Mare)	0·69
Fish Oils	Traces (p. 675)
Margarine	2·0–5·0
Vegetable Fats:	
Barley Germ Oil	63
Cocoa Butter	2·0
Coconut Oil	6·0–9·2
Corn Salad Oil	70
Cotton Seed Oil	35–50
Ground Nut or Arachis Oil	13–27
Linseed Oil	72–83
Maize Germ Oil	42
Oat Germ Oil	31
Olive Oil	4·0–13·7
Palm Oil	2·0–11·3
Rice Bran Oil	29–42
Rye Germ Oil	48
Soya Bean Oil	56–63
Sunflower Seed Oil	52–64
Wheat Germ Oil	44–52

Human Requirements of the Essential Unsaturated Fatty Acids Nothing is known about [illegible] though the Food and Nutrition Boar[illegible] the U.S.A. [61] *stated in 1948 t*[illegible] . . *it is desirable* . . . that the fat intake include essential unsaturated fatty acids to the extent of at least *one per cent. of the total calories* " Only about half this amount has been available in England since 1945. One human volunteer [58] has lived for six months on an almost fat-free diet, which produced the fat deficiency disease in rats, with no ill results, though, as might have been expected, he lost weight, his respiratory quotient altered and there was a marked fall in the level of linoleic and arachidonic acid in the blood. The frequent attacks of migraine from which he suffered were permanently cured. Of seven infants reported in the literature [8] who were given diets very *low* in fat, two developed eczema which was then cured by fat and there is some clinical evidence, discussed below, that eczema, especially *in infants, is* associated with abnormally low levels of unsaturated acids in the blood and *may be improved by adding unsaturated fatty acids to the diet. The condition* of the skin and especially of the hair in cœliac disease and other diseases where fat absorption is impaired [59, 60], in which the level of the unsaturated fatty acids of the serum is low [62, 63], may also possibly point to the unsaturated fatty acids being necessary in human nutrition.

But when it is remembered that rats show few definite symptoms of the fat deficiency disease until they have been several months on the deficient diet and when it is also remembered how high are the stores of the *essential* unsaturated fatty acids in man (p 672), it is *hardly surprising that neither* deliberately planned *deficient* diets *nor* diets *low in fat such as those in*

England cause any definite symptoms clearly ascribable to lack of the essential fatty acids

While however there appears to be no frank deficiency of these acids in human nutrition it is relevant to remember that in processed and stale food they may be deliberately destroyed to improve the keeping quality of the food or accidentally destroyed by rancidity while changes from the traditional feeding of cattle and poultry may alter the amounts in animal fats and dairy produce [4]

Essential Unsaturated Fatty Acids in Medicine

Eczema The only condition where these acids appear to have some definite value is eczema Hansen and his colleagues [62] have carried out the best and most extensive investigations on the part played by the essential unsaturated fatty acids in human disease and have reviewed the changes in the iodine number of the serum lipoids in various conditions [63] among which a rise in temperature and possibly dermatitis are of particular interest in their effect on lowering the level of unsaturated fatty acids fever has the same effect on the level of vitamin A (p 80) but not of vitamin E (p 602) An important point is that the iodine number should be estimated in serum or plasma and not whole blood as the latter at least in dogs [64] varies far less than the former when a deficiency of fat is present

One hundred and seventy one eczematous patients and one hundred and one controls taking the same type of diet had the iodine number of their sera estimated [65] Of the infants under two years of age eighty per cent had iodine numbers below the controls and so did seventy five per cent of children between the ages of two and fifteen years and over half the adults When lard in amounts up to three ounces daily or vegetable oils were given the response was good to excellent in sixty of one hundred and forty eight patients and fair to good in fifty one Most of those who did not respond were in the older age group Clinical improvement generally coincided with a rise in the serum iodine number This rise was also caused by crude coal tar ointments which is an argument in favour of it being the result and not the cause of the improvement in the condition of the skin but on balance it would seem that this is the wrong explanation Among the other investigators Cornbleet [66] gave eighty seven patients with allergic eczema most of whom were adolescents or young adults in whom the condition had been present for many years four tablespoonfuls three times a day of maize oil Cure took twelve to eighteen months and followed an erratic course concomitant asthma also often improved Faber and Roberts [67] confirmed the low iodine number in the serum lipoids of infants with eczema but could not confirm the curative properties of unsaturated oils Taub and Zakon [68] treated eczematous patients with raw linseed oil and reported that some were worse after the treatment while Ginsberg and others [69] failed to improve the eczema of infants and of adults with corn oil and linseed oil and found no difference in the iodine number of their serum lipoids compared to that of normal subjects The work of Finnerud and his colleagues [70] may explain these contradictory results since only half of forty seven eczematous patients had low iodine numbers and it appears that it was especially in all these patients that definite improvement occurred after taking three tablespoonfuls of lard daily which apparently were administered by being used in the cooking In the patients with normal serum values improvement was less constant The level of arachidonic acid in the blood was never low even when the iodine number was low

Burns and Wounds Cod liver oil is the oil which has been most extensively used in the treatment of burns and wounds but though it contains a large proportion of highly unsaturated fatty acids these are not to any marked degree the essential unsaturated fatty acids (p 671) Lohr and Zacher [71] first drew attention to the use of cod liver oil ointments for treating burns owing to the excellent results which they obtained in a

FIG 241 Infant with very severe eczema before and after treatment by mouth for eleven weeks with 200 mg daily of the ethyl esters of linoleic and linolenic acids combined with local applications of a two per cent ointment of these acids

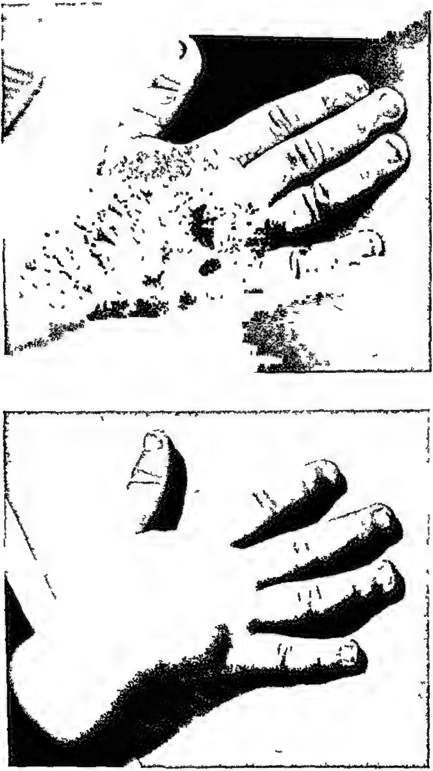

Fig 242 Hands of a child before and after the same treatment for fifteen weeks as that given to the infant shown in Fig 241 He had had eczema for two years on the back of the hands and behind the knees

large number of cases they especially stressed the rapid cleaning and epithelization Steel [72] confirmed these results both for burns and wounds being particularly impressed by the stimulation of indolent areas Dann and her collaborators [74] have carried out the most thorough experimental work yet published and have given an extensive bibliography to German clinical work They report that cod liver oil arachis oil and linoleic acid all stimulate granulation tissue though only the last stimulates epithelial regeneration Arachis oil causes excessive collagen formation Puestow and his colleagues [73] from excellent experimental work on pigs and rabbits concluded that burns treated with fish liver oils healed twenty five per cent more rapidly than burns which received no treatment or olive oil The effect was not due to the vitamins in the fish liver oils and it is interesting to note that the ' essential linoleic acid of the olive oil had no effect The bacteriostatic action of cod liver oil is said to be enhanced by its distillation at 250° C and its subsequent iodinization [75] a procedure which must destroy the unsaturated fatty acids whatever unknown substances are produced

Tuberculosis Here again it is cod liver oil and not the unsaturated vegetable oils which has won for itself a wide reputation in treatment It is difficult to understand why cod liver oil has this reputation it is more difficult to ignore it For over half a century physicians have been impressed by it both in England [76] and in America [77] and though at the present time it receives scant attention in medical journals yet when it is mentioned it is praised McConkey [77] for instance reporting after well controlled work that it gives remarkably good results in preventing laryngeal and intestinal tuberculosis in cases of pulmonary tuberculosis Emmerie and others [112] have isolated from cod liver oil an unsaturated fraction which at least *in vitro* inhibits the growth of tubercle bacilli The early French work on cod liver oil and lupus vulgaris and the present day work on large doses of vitamin D_2 and tuberculosis are discussed on p 5~2

OTHER MINOR FAT-SOLUBLE VITAMINS

Butter Growth Factor The review by Burr and Barnes [8] in 1943 gives an excellent brief summary of and references to the earlier conflicting evidence that butter fat contains a factor which promotes growth There can be little doubt that butter fat for the calf is superior to all the common vegetable oils and to a lesser degree to animal depot fats [78] For the rat which is the animal that has been most extensively studied Boutwell and his collaborators [~9] in their first papers reported that butter is superior to maize coconut cotton seed or soya bean oils this superiority apparently being due to the presence of long chain saturated fatty acids But in later papers these workers stated that the superiority of butter vanishes when mixed carbohydrates instead of lactose alone were given in the diet [80 81] On the lactose diets the flavour of the butter was not the factor which was responsible for the better growth and food consumption because removing the diacetyl—the substance which largely gives butter its distinctive flavour —from the butter did not decrease the effect of the butter nor was the relative effect of corn oil reversed by flavouring it with diacetyl [81]

Deuel and his collaborators [82] have confirmed that young rats prefer and consume far larger quantities of diets containing butter than diets containing vegetable oils They have also shown that vegetable oils flavoured with diacetyl are preferred to oils without this flavour but they have not confirmed or denied the crucial work of Boutwell and others [81] that even when vegetable oils are flavoured they are inferior to butter Their experiments [83] in which they showed that rats consuming the same amounts of diets containing butter or other fats grew equally well and had the same carcase composition are of little value since these by ensuring that the same

amount of food was eaten by all the rats, ignored the essential fact that stimulation of appetite is one of the most important properties of food and of vitamins such as aneurine. On the balance of evidence it would appear that butter, at least for calves and young rats, is superior to vegetable fats for promoting appetite and growth when lactose, the normal carbohydrate for young animals, is the only carbohydrate in the diet.

The factor in butter which stimulates growth is not [84, 85] as was once thought [86] vaccenic acid—an isomer of oleic acid with the double bond in the 11–12 position instead of the 9–10 position. But there is work [84, 87] which strongly suggests that some of the liquid fractions of butter contain the factor, especially when the pasture of the cow is good [84]. The physiological importance of the branched chain fatty acids of butter [111] has not yet been investigated.

Margarine's value for man compared to that of butter is of course of very great importance. So it is unfortunate—as it is in all work on nutrition—that the rat should be our guide when it is quite unsuitable to be so by its very capacity to thrive in abnormal laboratory conditions on grossly distorted diets. To some it might seem unwise to advise an army to march on a rat's stomach. On the other hand satisfactory human experiments are almost impossible: the only work [88] purporting to show that butter and margarine are equally valuable are completely vitiated because the two groups of children who eat the two different fats were living in two different institutions. The sole truly important evidence about margarine is given by the Ministry of Labour's investigation carried out before the war on the food consumption of nearly eleven thousand industrial and agricultural families [89]. An average of only three ounces per head per week was eaten compared to over six ounces of butter, though the latter was nearly three times as costly. In other words man seldom can stomach more than three ounces of margarine a week unless he is compelled by dire necessity.

Anti-Stiffness Factor. This factor, whose lack is reputed to cause chiefly stiffness of the wrists of guinea pigs and calcification of soft tissues, does not exist. The evidence against its existence is:—

(a) Some workers cannot confirm that it exists [90, 91].

(b) Stiff wrists in guinea pigs are common in normal animals, the condition waxing and waning for no known reason [92, 93].

(c) The calcification found by some [92, 96, 97] but not all [94] workers is probably due to poisoning by calcium or vitamin D caused by the excessive amounts of these included in the experimental diets.

(d) Even those who agree that the factor exists do not agree over what diets can demonstrate its deficiency [92].

(e) More than twenty-four widely differing steroids are said to cure the deficiency [93], including small amounts of vitamin D_2, though the experimental diets contain an abundance.

(f) There is complete disagreement as to what substances contain the anti-stiffness factor [92].

(g) Judging if animals are deficient by the stiffness of their wrists is a very crude and fallacious method [92] and not the delicate one it is claimed to be [95].

(h) A factor said to be active in doses of 0·00002 micrograms [105] is non-existent.

However, since some workers go on producing and discussing evidence about the action of the anti-stiffness factor it is necessary to discuss it as if it were no myth.

Bahrs and Wulzen [96] in 1936 noticed that planarian worms became diseased when fed on the tissues of guinea pigs whose vitamin C requirements had been supplied by tomato juice, orange juice or synthetic ascorbic acid. When, however, fresh green kale was used to provide vitamin C the tissues of the guinea pigs caused no disease in the worms.

Following up this indication that kale contained a new vitamin Wulzen and her collaborators [96, 97] showed that guinea pigs develop a definite deficiency disease when fed on a diet of grain and the "necessary" vitamins, or on a diet of skim milk supplemented with straw, orange juice, carotene and a salt mixture, in both cases very large amounts of vitamin D as irradiated yeast being given

The dominant symptom of the disease is stiffness of the wrists which gradually grows worse and also in time involves the elbows At autopsy the muscles are extremely atrophied and like those caused by lack of vitamin E [99] though they may be streaked with closely packed fine white lines of calcium deposits running parallel to the muscle fibres [96, 97] The heart muscle is normal [99] There are often lumps of calcium phosphate deposited under the skin in the region of the joints between the ribs and indiscriminately in many organs including the heart and aorta [96 97] This deposition of calcium phosphate appears to be due to the increase in the blood of both calcium and inorganic phosphorus [102] The serum phosphatase is decreased [102] and there is an abnormal distribution of the acid soluble phosphorus in the kidney and liver [103] and muscle [104] This disease is quite different to the muscular dystrophy caused by lack of vitamin E, since in animals suffering from both diseases either one can be cured separately and further, the "stiffness" disease, which does not cause creatinuria, is cured without reducing the creatinuria of the muscular dystrophy [98] The islets of Langerhans may be greatly enlarged [100] Deafness is common [101]

Cod liver oil accelerates the onset of the symptoms and aggravates the condition Neither the grass juice factor of Kohler, Elvejhm and Hart, nor vitamin E in th [illegible] *or prevents the* [illegible] to the contrary [illegible] steroids have been reported to be curative [93] ergosterol acetate being the most effective, though some workers give the necessary daily dose as 5 micrograms [93] while others [94] state 100 micrograms or more is needed *Calciferol though present in superabundance in the diet is said to be curative in small doses* [93]

Fresh kale or alfalfa and fresh raw cream, but not pasteurized, and sugar cane juice are the foods *par excellence* for the cure of the stiffness of the wrists according to Wulzen and her collaborators, the discoverers of the anti stiffness factor Thus from raw cream a *crystalline substance was extracted* which was fat soluble containing one carbonyl group and had a molecular weight of about 200 it was curative in doses of 0 1 micrograms daily [97] *But other workers [99] have found cream ineffective* From cane juice a factor active in 0 002 microgram [95] or even 0 00002 microgram [105] doses was extracted, yet cane juice in the hands of other workers is valueless [92] Stigmasterol has been acclaimed as the factor by two groups of workers [109]

Clinically the anti stiffness factor is valueless in scleroderma [106] and experimentally has no relation to the adrenal steroids and so probably would have no effect on rheumatoid arthritis [107]

Vitamin U or Anti-Peptic Ulcer Factor Cheney [108] is the only worker who has investigated this factor, but it appears probable that it exists Judging by the protective action against histamine produced peptic ulcers in guinea pigs, vitamin U is present in the fat of cabbage and probably in parsley, lettuce and celery, and eggs and raw milk It is very thermolabile and is rapidly destroyed by the wilting of vegetables

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CHAPTER X

VITAMIN K

HISTORY

BETWEEN 1929 and 1933 several observers described a hæmorrhagic disease in chicks fed on diets poor in fats. The existence of a vitamin deficiency as a cause of the hæmorrhage was suspected in 1929 by Dam [1] of Copenhagen, and later in 1931 by McFarlane and his collaborators [2, 3] in their work on the fat soluble vitamin requirements of the chick. At first it was thought that the hæmorrhagic syndrome was related to scurvy, although it is known that the chick synthesizes its own ascorbic acid. Cabbage, which contains this vitamin, was found to cure the h
of lemon juice, pure ascorbic aci
do so, showing that the condition
to any of the then known fat soluble vitamins [5]. In 1934 Dam [5] suggested that this hæmorrhagic tendency in chicks was a definite dietary deficiency disease due to lack of a fat soluble factor, and in the next year he proposed that this factor be called vitamin K (Koagulations Vitamin), a name that has been generally adopted.

The first definite proof of the fat soluble nature of vitamin K was supplied in 1931 by McFarlane and his co-workers, who noted that fish meal cured the hæmorrhagic disease in chicks on basal diets, but did not do so if the meal was first extracted with fat solvents. The fat soluble nature of the vitamin was conclusively proved by Dam [5], Almquist and Stokstad [6], who obtained crude concentrates of the vitamin by the extraction of certain foodstuffs with ether.

The relationship between the new vitamin and the clotting of blood was established when McFarlane [3] and Schonheyder [7] observed that the blood of chicks suffering from the nutritional hæmorrhagic syndrome had a prolonged clotting ... first suggested that the condition resulted ... was proved by Dam, Schonheyder and ... o isolate prothrombin from the blood of ... gh it was found to be present in the blood of normal chicks. They also noted that an aqueous emulsion of a vitamin K concentrate failed to restore clotting time to normal when added to a blood plasma thromboplastin mixture, thus showing that the prolonged clotting time was due to a low plasma prothrombin level in the vitamin K deficient chicks, and that vitamin K itself had no thrombin like activity. Quick [10] in 1937 observed a progressive fall in the prothrombin level in the blood of chicks on a vitamin K deficient diet. A distinct hæmorrhagic tendency appeared when low prothrombin levels were reached, and both the low prothrombin and the hæmorrhagic tendency were cured by the administration of foodstuffs rich in vitamin K.

Finally in 1937 it was shown that mammals and man may develop vitamin K deficiency with its associated hypoprothrombinæmia and hæmorrhagic tendency, not by feeding on a diet deficient in the vitamin, but in conditions associated with the absence of bile in the intestine. In the dog and rat this was achieved by an experimental biliary fistula [11, 12], in man it was observed in cases of obstructive jaundice and biliary fistula [13].

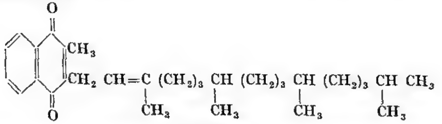
CHEMISTRY OF VITAMIN K

The early workers obtained concentrates containing crude vitamin K by extracting alfalfa and fish meal with fat solvents Long before it was isolated the general chemical properties of the vitamin were known Thus it was found to be fat soluble, alkali labile, inactivated by light in a few hours, and destroyed by oxidizing agents and strong acids Vitamin K in concentrates of alfalfa or of hog liver fat resists temperatures of 100°–120° C for twenty four hours It can be concentrated by adsorption on a number of substances, including acidic fuller's earth synthetic zeolites (permutit), calcium sulphate and activated carbon, from which it may be eluted with fat solvents such as petroleum ether Crude preparations can be concentrated by molecular distillation or chromatographic adsorption using dehydrated magnesium sulphate and then zinc carbonate

Following Almquist and Stokstad's discovery in 1935 that alfalfa (lucerne) meal is a potent source of vitamin K [6] numerous attempts were made to isolate it from this substance by utilizing certain properties of the vitamin, such as differential solubility, adsorption and elution, and molecular distillation The isolation of vitamin K in pure or almost pure form was first achieved in January, 1939, by Karrer, Dam, and their associates [16], who obtained it as a yellow oil Binkley [20] and his co workers obtained vitamin K in the form of yellow rosettes with a melting point of − 20° C

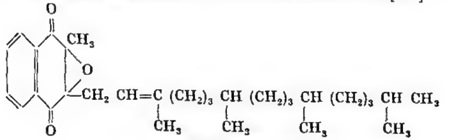
By the spring of 1939 it became apparent that more than one compound possessed vitamin K activity, and that there existed a number of analogues closely resembling the vitamin K isolated from alfalfa Thus MacCorquodale [17] McKee [18] and co workers isolated two different compounds from alfalfa meal and putrefying fish meal respectively They named the vitamin obtained from alfalfa vitamin K_1 and that from fish meal vitamin K_2 Almquist and Klose [19] also found that phthiocol, a compound originally isolated from tubercle bacilli had a slight vitamin K action

Within a short time of the isolation of vitamin K_1 from alfalfa its structure was identified, as a result of the work of Doisy [20] Almquist and Klose [21] Fieser and his collaborators [22] It is 2 methyl 3 phytyl 1 4 naphthoquinone—



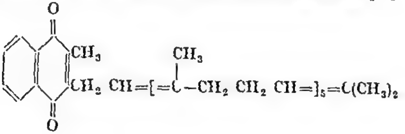
Vitamin K_1

The final step in the proof of this structure was the synthesis of the vitamin in three laboratories practically simultaneously a few months later [23–25] Vitamin K_1 oxide, unlike the parent substance is unacted upon by light, and is as active It has been used clinically It is insoluble in water, but a suspension may be injected by drawing up a solution in alcohol (10 mg in 3 ml) in a syringe and diluting with 10 ml of normal saline [268]



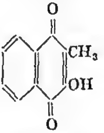
Vitamin K_1 Oxide

Vitamin K_2 which was isolated from putrefying fish meal by methods similar to those used for the isolation of vitamin K_1 is a light yellow crystalline solid melting at 53 5° to 54 5° C with a biological potency of about two thirds that of vitamin K_1 It is a 2 methyl 3 difarnesyl 1 4 naphthoquinone with an unsaturated hydrocarbon side chain [26]



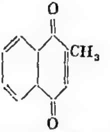
Vitamin K_2

Synthetic Analogues of Vitamin K Many compounds related to vitamin K_1 are known to have an antihæmorrhagic action The first vitamin K analogue to be discovered was phthiocol, or 2 methyl 3 hydroxy 1 4 naphthoquinone

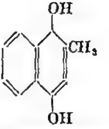


Phthiocol

Unlike vitamins K_1 and K_2 it is water soluble, and forms yellow prismatic crystals melting at 173° C Most of the analogues are derivatives of 1 4 naphthoquinone or 1 4 naphthohydroquinone A synthetic naphtho quinone that has been exhaustively studied is 2 methyl 1 4 naphtho quinone which is official in the B P C as menaphthone and in the U S P as menadione



2 Methyl 1 4 naphthoquinone (Menaphthone Menadione)

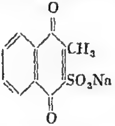


2 Methyl 1 4 naphthohydroquinone

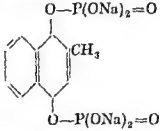
This has a high antihæmorrhagic potency, and weight for weight it is in man stated to be more potent than vitamin K_1 [176] although according ... Methyl 1 4 naphthoquinone ... is impaired by exposure to ... ngth of time It is absorbed when applied cutaneously in a fatty base [189] but is not suitable for intravenous use because of its oily character

A number of derivatives of 2 methyl 1 4 naphthoquinone have been prepared 2 Methyl 1 4 naphthohydroquinone diacetate (acetomenaphthone B P C) is more stable than 2 methyl 1 4 naphthoquinone, but has the ... The sodium salts of 2 methyl 1 4 ... id ester [28] and 2 methyl 1 4 ... 169 246] and menadoxime the ammonium salt of 2 methyl naphthoquinone 4 oxime *O carboxymethyl*

ether, are water-soluble and stable They are used for intravenous injection



Sodium salt of 2 methyl 1 4 naphtho quinone 3 sulphonic acid



Sodium 2 methyl 1 4 naphthohydroquinone diphosphoric acid ester

It has been shown in both the experimental animal and in human beings that water soluble vitamin K analogues are absorbed from the gastro intestinal tract without the aid of the bile salts originally given with natural vitamin K and its fat soluble analogues [50, 51] Smith and Owen [51] have shown that 4 amino 2 methyl 1-naphthol (vitamin K_5), which is water soluble, is physiologically active without bile salt medication 4 Amino 2 methyl
are stated to be
quinone [64, 65]
gram positive and
that have been used
[148], 2 methyl 1 4 naphthohydroquinone bisulphite and 2 methyl 1 4-naphthohydroquinone dibutyrate [332]

These compounds are all of comparable activity The following table shows the activity of some of them in terms of that of menaphthone (1,000) based on a biological assay

Menaphthone	1,000
Acetomenaphthone	450
Vitamin K_1	300
Vitamin K_2	240
2 Methyl 4 amino 1 naphthol hydrochloride	470
Sodium salt of 2 methyl 1 4 amino naphtho hydroquinone diphosphoric acid ester	490

FIG 213 Crystals of Menaphthone (2 Methyl 1 4 naphthoquinone)

An intact benzene ring in the vitamin K molecule is essential for activity if it is substituted activity is lost The 2 methyl group is also essential and cannot be replaced by hydrogen or by other groups without serious loss of activity The quinone structure is not essential, e g, the hydroquinones are almost as active, and the quinone oxygen can be replaced by other groups such as amino and aldehyde The side chain in the 3 position can be eliminated without loss of activity

Natural vitamin K and its synthetic analogues have been estimated by (a) reduction and titration with 2 6 dichlorophenolindophenol, using phenosafranine as an indicator, or with ceric sulphate, with o phenanthroline as indicator [238 239], (b) direct titration with excess bromine in carbon tetrachloride, the excess bromine being titrated by means of potassium iodide and sodium thiosulphate solution [239] A colorimetric test using sodium diethyl dithiocarbamate has also been devised [240]

UNITS OF VITAMIN K

The methods used for the assay of vitamin K and its analogues are based upon changes in the clotting time or upon the prevention of hæmorrhage Units were devised by Schønheyder [29] Dam and Glavind [30] Thayer and Doisy [31] Ansbacher [32] and Dann [33] Almquist and his co workers [34 35] also developed a method of assay These units are now obsolete owing to the use of menaphthone and its analogues in both biological and clinical work No adequate experimental data are available from which the various units can be correlated The following equivalents are approximate only —

1 Ansbacher unit = 20 Dam units
1 Thayer Doisy unit = 30 Dam units
1 Almquist Stockstad unit = 37 5 Dam units
1 Dann unit = 25 Dam units
1 gram vitamin K_1 = 12,000,000 Dam units

This table is given because in much of the earlier work dosage of vitamin K is given in units 1 mg of pure vitamin K_1 is equipotent with 450μg of menaphthone

DISTRIBUTION OF VITAMIN K IN FOODS

Green plants are the richest sources of vitamin K, although moderate amounts are found widely distributed in the animal body Alfalfa (lucerne) and spinach are rich sources of the vitamin Other plants with a fairly high vitamin K content are cauliflower, cabbage carrot tops, kale soya bean chestnut leaves pine needles and seaweed It is also present in tomatoes hemp seed, bran and orange peel The green parts of the plant contain more than the fruits, seeds and roots Most fruits except tomatoes are poor sources of vitamin K Mountain ash berries and honey contain the vitamin or at any rate an anti hæmorrhagic principle [15 313] Vitamin K *is also* found widely distributed throughout the animal body Dam [39] and his colleagues examined chicks on a normal diet and found that the vitamin was distributed in relatively large amounts in all tissues, liver and lung contain the least Apparently increased stores of vitamin K are found in the tissues *of animals receiving a diet rich in the vitamin* The best animal sources of vitamin K never contain more than ten per cent of that present in alfalfa Egg yolk contains small but variable amounts of the vitamin According to Dam [40] human urine contains no vitamin K, even after the consumption of diets rich in the vitamin

Bacteria can synthesize vitamin K It has been shown that 0 6 to 2 0 gm of dried bacteria per kilo of basal diet will protect chicks against vitamin K

deficiency, some bacteria are as potent in their vitamin K content as alfalfa Fæces are rich in the vitamin, its production being attributed to bacterial action although Andrus [153] from experiments on isolated intestinal loops disputes this Moulds, yeast and fungi contain practically no vitamin K

The vitamin K content of some materials, based on dry weight, is given below —

	Dam Units per 100 *grams*
Alfalfa	20,000 to 40,000
Algæ	13 000 to 17,000
Cabbage leaves	40 000
Cauliflower	40 000
Chestnut leaves	80 000
Fæces	30 000
Liver (pork)	5,000 to 10 000
(poultry)	300
Maize (leaves)	1 400 to 1,800
Nettle leaves	40 000
Pine needles	20 000
Putrefied fish meal	90 000
Spinach leaves	55 000
Tomato green	10 000
ripe	5 000
Carrots	1,000
Cereals	500 to 4 000
Eggs from chickens fed on diets rich in alfalfa	1,000
Fish meal	500
Milk, cows'	very little
Milk, human	0 to 200
Parsley	200
Peas fresh	3 500
Potatoes	1,000
Rose hips	2,800
Strawberries	2,250

PHYSIOLOGY OF VITAMIN K

Function Vitamin K is essential for normal blood coagulation The term "anti hæmorrhagic vitamin" for vitamin K is misleading It does not arrest hæmorrhage in normal persons or in hæmophilia, purpura or bleeding diseases As far as is known vitamin K has only one function in the body It participates in an enzyme system in the liver to form prothrombin, the precursor of thrombin, a water soluble glycoprotein present in plasma to the extent of 15 to 20 mg per 100 ml [386], it is ineffective *in vitro* if added to prothrombin deficient plasma or blood Vitamin K does not form part of the prothrombin molecule but probably serves as the prosthetic group which complements the apo enzyme in the enzyme system synthesizing prothrombin [418] According to Quick [270] prothrombin is composed of calcium and two separable components one of which (A) is stable and appears to be related to the oxidation reduction systems of the blood, the other (B) being heat labile and inactivated by dicoumarol (p 716) Prothrombin A, which is stated to be present partly free and partly combined in plasma, is said to be diminished in vitamin K deficiency and in stored plasma [390] A "labile factor" that is destroyed at 58° C and disappears from stored plasma due to slow oxidation is also stated by Quick to be essential for prothrombin activity It is not inactivated by dicoumarol [392] Quick's prothrombin A is probably identical with his "labile factor," and with

Ac globulin (p 692) and the Factor V of Owren [271] Lack of Owren's factor produces a hæmorrhagic diathesis termed *parahæmophilia* [271] which differs from true hæmophilia in that blood coagulation does not return to normal by the addition of thromboplastin Parahæmophilia manifests itself by hæmorrhage into the skin epistaxis menorrhagia and hæmaturia Owren [348] has recently prepared another factor Factor VI which is formed from V Seegers and co workers [263] reject the multiple component hypothesis of Quick and Owren as they have prepared prothrombin that is electrophoretically homogeneous They state it is a pure protein and that it can be activated to thrombin by sodium citrate It has been suggested that vitamin K takes part in an oxidation reduction system in which SH groups are oxidized to S S which has been postulated to occur in the transformation of fibrinogen to fibrin [20] Stefanini [414] has devised a method for determining the labile factor and finds that it is depleted in liver dysfunction the thrombocytopenia of acute leukæmia the hypoprothrombinæmia of terminal carcinoma and the immediate post operative period

The coagulation of the blood results *from a series of complex reactions* involving the interaction of prothrombin thromboplastin (thrombokinase) calcium thrombin and fibrinogen * *There is also an opposing mechanism* the inhibition of coagulation in which plasma antiprothrombin plasma *antithrombin and heparin play a part Heparin interferes with the conversion* of prothrombin to thrombin lessens the tendency of platelets to agglutinate catalyses the antithrombic activity of plasma and may inhibit the activity [illegible] d clots the soluble plasma [illegible] of the clot by the action [illegible] *in the blood* but is only produced when blood is shed by the *interaction* of prothrombin calcium ions and thromboplastin Calcium and prothrombin are both normally *present in blood plasma* thromboplastin probably exists in circulating plasma in an inactive form thromboplastinogen which *in the presence of an enzyme derived from platelets changes to active thromboplastin* According to Quick [161] *the action of calcium is stoichiometric and not catalytic*

The clotting of blood has been formulated as a two stage process for about half a century

Prothrombin + labile factor + calcium + thromboplastin = thrombin
Thrombin + fibrinogen = fibrin clot

The reaction between these factors is *stoichiometric* according to Quick and catalytic according to Ware and Seegers [8] A number of recent studies suggest that there are several other new factors involved in the conversion of prothrombin to thrombin Ware and his co workers [187] *have prepared* a blood globulin (*accelerator globulin or Ac globulin*) that accelerates the conversion of thrombin to prothrombin It decreases in experimental liver damage in dicoumarol therapy and slowly in citrated human plasma after ten days storage It is increased in patients taking large doses of aminophylline and in patients with thrombo embolic disease showing evidence of intravascular clotting [409] *Ac globulin may be identical with* Owren's *Factor V Quick's labile factor and the* accelerator factor of Fantl and Nance [413] These factors are collectively referred to by Stefanini [417] as "plasma prothrombin conversion factor" (P P C F) Alexander and others [404] have described a serum factor they call S P C A (Serum prothrombin *conversion accelerator*) which accelerates the conversion of prothrombin to thrombin It has been isolated and may be identical with thrombokinase [406] A *lipoid substance also seems to be essential for clotting since fat free plasma will not clot on the addition of a fat free thromboplastin and calcium ions*

* *The complexity of the subject may be judged by the fact that 10 000 papers on this subject have been written*

Ware and Seegers [265] have formulated the following scheme to explain the reactions occurring during the clotting of blood.

First Phase
Prothrombin + Thromboplastin + Calcium = Thrombin (in small amounts)
Plasma Ac globulin + Thrombin (in small amounts) = Serum Ac-globulin.
Prothrombin + Thromboplastin + Calcium + Serum Ac-globulin = Thrombin (large amounts)
Second or Active Phase
Thrombin + Fibrinogen = Fibrin

It was formerly thought that calcium in ionized form was indispensable for the clotting of blood, but Quick [270] has recently produced evidence that the calcium is intimately linked with the prothrombin molecule The reaction between prothrombin, thromboplastin and calcium occurs between pH 6 2 to 8 7 [373] According to Loomis and Seegers [373] calcium can be replaced by related metals such as strontium

In mammals prothrombin is present in large excess over minimal needs for efficient clotting. Thus in dogs the prothrombin may be reduced to one-fifth of the normal level without prolonging the clotting time or bleeding time In human beings normal plasma only contains about twice as much prothrombin as is needed to prevent the development of a hæmorrhagic tendency The speed at which blood clots not only depends on the level of prothrombin in the blood, but also upon its "convertibility" Increased convertibility of prothrombin may compensate for a relative deficiency in amount [42]

The liver is the site of prothrombin formation [110] Extensive hepatic damage in the human being is associated with hypoprothrombinæmia [43], and in the experimental animal hepatic toxins, such as phosphorus, chloroform, carbon tetrachloride, hepatic tumours [374], and hepatectomy cause a fall in the blood prothrombin [44, 318] If the hepatic injury is severe enough the administration of vitamin K is not effective in correcting the prothrombin deficiency [45] With regeneration of the liver, the blood prothrombin returns to normal levels. Chloroform, which is a well known hepatic poison produces a fall in the plasma prothrombin level of human beings [46] Adrenaline on the other hand [191] causes a rise in the prothrombin level [191]

Busing and Zuzak [345] have shown that the complement titre in young chicks parallels the vitamin K intake On a low intake the titre is consistently low, it rises with increased vitamin K intake and is higher in chicks receiving an excess of the vitamin than in normal birds

The fate of plasma prothrombin after its formation in the liver and release into the circulating blood has been the subject of experimental work [47]. Certain studies point to the lungs as the site of disappearance of plasma prothrombin A possible explanation of the *rôle* of the lungs is thought to be the production of blood platelets in this organ [48] Platelets undergo disintegration and initiate the first stage of the clotting process by releasing thromboplastin, which, in the presence of calcium, changes prothrombin to thrombin Prothrombin is present in the bone marrow [135]

Absorption of Vitamin K. Bile is essential for the proper absorption of natural vitamin K Synthetic vitamin K analogues, particularly the water soluble ones, do not need bile salts for their absorption, if given in adequate dosage (p 703) The exact point of absorption in the gastro intestinal tract is not known for certain Butt and Snell [179] believe that it is not absorbed through the colon or lower part of the ileum, but from the upper part of the small intestine. The taking of mineral oil (liquid paraffin) inhibits the proper utilization of vitamin K. The oil is not absorbed and

dissolves the vitamin K, most of which is voided in the oil with the fæces [172, 190, 212, 213].

Storage of Vitamin K. Vitamin K is stored in the liver in small but definite amounts [49]. The storage cannot be appreciable because fatal hypoprothrombinæmia can occur in a week. Studies with menaphthone containing radio-active carbon (C^{14}) show that only traces are present in the blood [435].

Excretion of Vitamin K. Vitamin K is present in relatively large quantities in the fæces, being mostly derived from the bacterial flora inhabiting the intestine. These are known to synthesize vitamin K, e.g., *E. coli* contains 750 to 1,600 Dam units per gram of dry weight, and it can synthesize the vitamin from a simplified medium containing asparagin, glucose and citrate. It is not known to what extent vitamin K taken in the food is excreted.

Pharmacology. No toxic effects have been observed clinically after the administration of vitamin K or its analogues in therapeutic doses. Doses up to 200 mg. of 2-methyl-1 : 4-naphthoquinone are well tolerated [277] and 40 mg. of 2-amino-2-methyl-1-naphthol hydrochloride have been given intravenously for a period of nineteen days without any untoward effects [179]. Eight mg. of menaphthone has been administered daily for thirty months without untoward effects [277].

Excessive doses of vitamin K or its analogues are toxic to the experimental animal. The oral lethal doses (L.D.$_{50}$) of phthiocol and 2-methyl-1 : 4-naphthoquinone for mice are 200 and 500 mg. per kilogram respectively. The figures for the sodium salt of 2-methyl-1 : 4-naphthohydroquinone diphosphoric ester and 2-methyl-1 : 4-naphthohydroquinone sodium bisulphite are of the same order [275]. Vitamin K_1 is not lethal in doses of 25 mg. per kilogram [58]. The oral toxicities of the vitamin K analogues are approximately one-third to one-fifteenth of their subcutaneous toxicities. Chronic toxic effects are due to injury to the circulating red cells [58, 274], and aplastic anæmia [275]. In toxic doses the compounds produce respiratory depression and acute vascular congestion. As the maximum single therapeutic dose of any of the vitamin K analogues appears to be about 20 mg. there is no danger of untoward reactions from doses of this order.

Russell and Page [214] have shown that 2-methyl-1 : 4-naphthoquinone is absorbed through the skin and that the application of 10 mg. of the substance in an ointment protects newborn infants from hypoprothrombinæmia. Vollmer and co-workers [276] have shown that the rate of percutaneous absorption is enhanced by decreasing the viscosity of the solvent. They find doses of 0·1 to 1 mg. effective in four hours. This method of administration, which is of academic rather than practical interest, is not without danger as it may lead to dermatitis [215].

Menaphthone intramuscularly produces a lowering of the blood pressure in hypertensive rats [356] and in patients with hypertension [36]. In this respect it resembles the action of some other quinones.

If added to whole blood menaphthone causes marked methæmoglobin formation [357].

Massive doses of vitamin K, e.g., 20 to 75 mg., produce hyperprothrombinæmia in animals and man, which is also said to be produced by theophylline, theobromine and caffeine [380, 381], although the latter statement has been questioned [41]. There is no evidence that the use of such drugs clinically, appreciably alters the coagulation time of blood [370].

The vitamin K analogue, tetrasodium 2-methyl-1 : 4-naphthohydroquinone diphosphate produces mitotic inhibition in chick fibroplast cultures and in some human carcinomata [236].

REQUIREMENTS OF VITAMIN K

The human requirements of vitamin K are not known. Bacterial synthesis of the vitamin can occur in the large intestine (p. 690), but whether adult

man needs an exogenous source is unknown. It is assumed that he does, as dietary vitamin K deficiency in man has been reported [52, 53]. According to Ponchet [360] the vitamin K produced in the intestine by bacterial action is an important endogenous source in the human being, and is probably more important than exogenous sources. Bile is essential for the absorption of vitamin K formed in this way. The newborn is considered to require an exogenous source as the prothrombin level falls after birth and only returns to normal levels after breast or artificial feeding. Normally the newborn infant gets from almost nothing to 400 Dam units of vitamin K_1 (= 33 micrograms) from mothers' milk daily [298]. Hardwicke [299] found the minimal effective dose of a vitamin K analogue (tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphoric ester) necessary to prevent the development of hypoprothrombinaemia in the newborn infant. This was 0·5 to 5 micrograms daily. In established hypoprothrombinaemia of the newborn a dose of 1·25 mg was sufficient to restore the prothrombin level to normal. Sells, Walker and Owen [38] consider that the vitamin K requirement of the newborn is 1 to 2 micrograms daily, an amount provided by the milk. If adults require an exogenous source of vitamin K this would be something less than 0·1 mg daily on the basis of the figures of Sells, Walker and Owen.

According to Mills, Cottingham and Mills [300] a rise in environmental temperature increases the requirements of vitamin K in the experimental animal. Animals adapted to tropical heat seem more prone to severe manifestations of vitamin K deficiency than controls kept under temperate conditions.

VITAMIN K DEFICIENCY

Vitamin K deficiency, which is detected by a lowering of the blood prothrombin level (hypoprothrombinaemia) may occur in any of the following circumstances:

Inadequate Supply of Vitamin K. This may be due to the following causes:

(a) *Nutritional Deficiency of Vitamin K.* That a nutritional vitamin K deficiency can exist is well supported by the experimental production of low prothrombin levels in chicks, rabbits, rats and mice by the administration of diets deficient in vitamin K [54–56]. Apparently the vitamin formed by bacterial synthesis in the intestine is insufficient for normal requirements.

Kark and Lozner [52] observed four patients with a lowered blood prothrombin level unassociated with disease of the liver or biliary tract and due apparently to a simple dietary deficiency. Three had scurvy and one an alcoholic suffered from pellagra and subclinical scurvy. Vitamin K without bile salts was given by mouth and the day after the plasma prothrombin level returned to normal showing that a nutritional deficiency of vitamin K was the cause of the hypoprothrombinaemia.

Scarborough [53] has reported eighteen cases of nutritional deficiency of vitamin K in patients without any clinical evidence of hepatic or biliary tract disease. An examination of the diet and laboratory tests showed that all patients were deficient in one or more vitamins.

Vitamin K deficiency is not common in pellagrins, but complicating factors such as prolonged diarrhœa may result in a serious deficiency of this vitamin according to Warner and his co-workers [164]. They believe that many chronically debilitated persons have a moderate hypoprothrombinæmia which is probably due to inefficient utilization of vitamin K, rather than to a lack of it.

A deficiency of vitamin K has been recorded in anorexia nervosa [301].

(b) *Conditioned Deficiency of Vitamin K.* A conditioned deficiency of vitamin K may be produced by certain drugs. Thus sulphaguanidine and succinylsulphathiazole and other sulphonamides, such as sulphadiazine

sulphathiazole and sulphaquinoxaline [146], administered to rats produce a fall in the blood prothrombin, probably by interfering with the bacterial synthesis of the vitamin in the gut of the animal [302] The hypoprothrombinæmia can be prevented by vitamin K analogues [379] Hæmorrhage responding to vitamin K has been recorded in patients given succinylsulphathiazole [306] and phthalylsulphathiazole [388] It is also stated that the [illegible] [illegible]evels of infants given sulphathiazole According to Pirk and Engelberg [359] quinine in therapeutic doses causes a fall in the blood prothrombin level, reversed by large doses of vitamin K, although this effect of quinine is denied by Quick [221] The discrepancies are probably due to differences in the procedures employed for determining prothrombin

The administration of propylthiouracil for hyperthyroidism is stated to have caused hypoprothrombinæmia, due to deficiency of accelerator globulin, a case described responded to treatment with serum and blood [419]

Huebner and Link [305] have shown that dicoumarol, which produces a hypoprothrombinæmia, can be degraded to salicylic acid It has been shown that salicylic acid and aspirin can induce hypoprothrombinæmia, which can be prevented by vitamin K [303, 304] It is now considered that the prolonged prothrombin time resulting from the administration of salicylates, as in rheumatic fever, is of no clinical significance in most patients and only approaches a critical level likely to precipitate hæmorrhage in very few patients [231] Approximately 1 mg of menaphthone counteracts the prothrombinopenic activity of 1 gram of aspirin in man [319] Jaques and Lepp [244] consider that salicylates and aspirin might be converted into dicoumarol in the gut by bacterial action

Some drugs, e g penicillin, streptomycin, aureomycin, the mercurial diuretics (e g me[illegible]als, amphetamine and methylamphetami[illegible] blood [426]

(c) *Idiopathic* [illegible] hypoprothrombinæmia has been describe[illegible]d by Rhoads and Fitz-Hugh [228] the s[illegible] male with a hæmorrhagic diathesis, due to [illegible] was no evidence of dietary vitamin K defici[illegible]rded a family incidence of idiopathic hypoprothrombinæmia with evidence that it may be associated with some functional liver defect, and Quick [372] has described a congenital and familial hypoprothrombinæmia due to lack of component B, and which is refractory to treatment with vitamin K. Another type of congenital hypoprothrombinæmia due to lack of component A and Factor V has also been described [390, 399] Hagen and Watson [188], who observed a case over a decade, describe the occurrence of epistaxis, subcutaneous hæmatomata, hæmorrhage into joints, menorrhagia and metrorrhagia The only agents effective in preventing the hæmorrhage were purified prothrombin and human plasma

(d) *Hypoprothrombinæmia of the Newborn* In the newborn infant and during the first few days of life there is a deficiency of prothrombin in the blood, and an increased tendency to hæmorrhage [57] This subject is further discussed on p 708

Inadequate Intestinal Absorption Inadequate intestinal absorption of vitamin K may result from —

(a) Lack of bile in the intestine due to defective secretion of bile salts, as in infective jaundice, or due to biliary fistula and operations on the biliary tract

(b) Obstructive jaundice of all types (e g , due to stones, cancer, stricture) Bile fails to reach the intestine, and ingested vitamin K is not absorbed

(c) Pyloric and intestinal obstruction [63]

(d) Pancreatic Insufficiency Pancreatic achylia and pancreatectomy in

the decrease following a hyperbolic curve ibin time is also directly proportional to the is kept [324]

One-Stage Method Two methods have been sma the other whole blood

This was developed by Quick [68] and investigators Quick s method is a measure the conversion of prothrombin into thrombin sion of fibrinogen into fibrin by this thrombin ctivity of prothrombin and the labile factor d consists of determining the clotting time of after the addition of an excess of a preparation ed amount of calcium chloride With a given the normal plasma prothrombin time is practic pecies for normal human beings using Quick s) 12 5 seconds which represents one hundred per thrombin The thromboplastin preparation used btained from rabbit brain others have used an] Most workers use dried extracts of brain A thromboplastin preparation is now available en and Toohey [193] simplify the process by using prothrombin time for plasma containing as little as nbin is nearly the same as for normal plasma so that time does not necessarily mean that the plasma con rothrombin This difficulty has been overcome by ibin times not only on whole plasma but also after] Non soapy detergents e g Teepol should not be sware as traces interfere with the estimations [428]

itions of the Quick method have been made Thus [70] studied the effect of adding varying amounts of er a wide range and found that which gave the lowest th optimal recalcification this was ten seconds for human has also been used to prevent coagulation instead of calcium nical purposes the use of tissue extracts as a source of as several disadvantages since such extracts are tedious to tency varies and they may interfere with observations on the fibrin web when the plasma clots Fullerton [71] Page 192] have therefore used a preparation of Russell viper

otained by Quick s original method The addition of lecithin tentiate the action of viper venom so that normal prothrombin i six to ten seconds [72 192] the activity of the lecithin depends sence of a fat soluble factor [241] The one and two stage not give the same results although if Russell viper venom is ne stage method the re of the two techniques are more in 75]

iodifications of the Qu od have been made by Holmboe i stable thrombopl iration from sheep brain by d Weinstein [242] [178] who uses lung extract as thromboplastin s a mechanical shaker for the rease the point of the coagulation time as iod by using prothrombin free supply (p 692) in the coagulation

] and] have shown that greater

a hæmorrhagic encephalitis following massive arsenotherapy in syphilitics, but the prothrombin level of the blood was not recorded.

In a series of patients with liver disease, studied by Herbert [314], sixty-eight per cent. had hypoprothrombinæmia. If, however, the hepatic parenchyma is damaged or there is extensive liver disease, vitamin K is ineffective in restoring the prothrombin level. Herbert also states that patients with a normal plasma prothrombin level may develop hypoprothrombinæmia and bleed a few days after operation.

Infarction of the liver is associated with hypoprothrombinæmia [315].

Infection, particularly of the Respiratory Tract. There is increased destruction of prothrombin in artificial fever therapy, and prolonged fever, particularly pneumonia [167].

Tocantins and Hause [225] note that hypoprothrombinæmia is observed regularly in pneumonia, especially in the first stages of the disease. This diminution in prothrombin no doubt results from a disturbance in hepatic function and may account for the delayed blood coagulability and the moderate hæmorrhagic manifestations observed in pneumonia. There are other evidences of liver dysfunction in pneumonia, such as hyperurobilinuria and diminished response to liver function tests. The slowness of the return of the prothrombin level to normal after the acute stage of pneumonia and the poor response to vitamin K therapy are also evidence of liver dysfunction. Hypoprothrombinæmia in pneumonia may also be due to increased utilization of prothrombin in the fibrinous exudates of the lung. Nja [92] has described a case of allergic purpura with hypoprothrombinæmia associated with pneumococcal infection, which showed a reduction in prothrombin time from five minutes to twenty seconds after treatment with sulphathiazole.

A number of observers have recorded that patients suffering from active and chronic pulmonary tuberculosis generally show low prothrombin levels [226, 227, 237, 320–322]. Patients with active disease or hæmorrhage show a more marked diminution in the prothrombin level than chronic or healing cases. Hæmoptysis cannot, however, be predicted from the prothrombin level [320]. The prothrombin time in tuberculous patients is restored to normal by giving vitamin K, unless the condition has so progressed that there are signs of hepatic damage. Vitamin K has no effect on pulmonary hæmorrhage [235, 320]. These conclusions are contested by Plum and Poulsen [323], who state that the prothrombin level of tuberculous patients, even in those with hæmoptysis, is within the normal range.

As in the case of pneumonia the lowered prothrombin level is probably related to the toxæmia resulting from tuberculous infection.

Hæmorrhage. It is possible that the prothrombin level might fall after a massive internal hæmorrhage, particularly if fluids and not blood are given to the patient to restore blood volume. These would dilute the blood and some time might elapse before sufficient prothrombin is made in the liver. Coller and Farris [62] have recorded a case of massive gastric hæmorrhage in which a low prothrombin level was found after the intravenous administration of 7·5 litres of fluid.

It is stated that the prothrombin time is well below normal in patients during an attack of acute coronary occlusion [371] and in heatstroke [143].

TESTS FOR VITAMIN K DEFICIENCY

Vitamin K is not estimated as such, but the prothrombin level of the blood is found by a clotting method. The prothrombin level should be determined as soon as possible after the blood is obtained from the patient, as the value changes with storage. Page and De Beer [324] have shown that at a given temperature the prothrombin time increases as a straight line function of the logarithm of the storage time ; Kove and Benton [405].

however, state that it decreases the decrease following a hyperbolic curve The rate of increase of prothrombin time is also directly proportional to the temperature at which the blood is kept [324]

Prothrombin Determination One-Stage Method Two methods have been devised one using oxalated plasma the other whole blood

Plasma Prothrombin Time This was developed by Quick [68] and modified later by a number of investigators Quick s method is a measure nt of the time necessary for the conversion of prothrombin into thrombin for the subsequent conversion of fibrinogen into fibrin by this thrombin stimates the continued activity of prothrombin and the labile factor 691) Briefly the method consists of determining the clotting time of ated plasma at 37 5° C after the addition of an excess of a preparation thromboplastin and a fixed amount of calcium chloride With a given thromboplastin preparation the normal plasma prothrombin time is practically constant for any one species for normal human beings using Quick s original method it is 11 to 12 5 seconds which represents one hundred per cent concentration of prothrombin The thromboplastin preparation used by Quick was cephalin obtained from rabbit brain others have used an extract of beef lung [178] Most workers use dried extracts of brain A standardized rabbit lung thromboplastin preparation is now available commercially [416] Owen and Toohey [193] simplify the process by using a saline extract The prothrombin time for plasma containing as little as sixty per cent prothrombin is nearly the same as for normal plasma so that a normal prothrombin time does not necessarily mean that the plasma contains 100 per cent prothrombin This difficulty has been overcome by determining prothrombin times not only on whole plasma but also after serial dilutions [52 69] Non soapy detergents, e g Teepol should not be used for cleaning glassware as traces interfere with the estimations [428]

Various modifications of the Quick method have been made Thus Pohle and Stewart [70] studied the effect of adding varying amounts of calcium chloride over a wide range and found that which gave the lowest clotting time With optimal recalcification this was ten seconds for human plasma Heparin has also been used to prevent coagulation instead of calcium chloride For clinical purposes the use of tissue extracts as a source of thromboplastin has several disadvantages since such extracts are tedious to prepare their potency varies and they may interfere with observations on the formation of the fibrin web when the plasma clots Fullerton [71] Page and others [162 192] have therefore used a preparation of Russell viper venom (*e g* Russven Stypven) in place of other thromboplastin preparations Normal prothrombin time using viper venom varies from eighteen to twenty five seconds When viper venom reagents are used to estimate prothrombin in plasma from patients receiving dicoumarol the results are much higher than those obtained by Quick s original method The addition of lecithin appears to potentiate the action of viper venom so that normal prothrombin times are from six to ten seconds [72 192] the activity of the lecithin depends upon the presence of a fat soluble factor [241] The one and two stage methods do not give the same results although if Russell viper venom is used in the one stage method the results of the two techniques are more in agreement [375]

Further modifications of the Quick method have been made by Holmboe [73] using a stable thromboplastin preparation from sheep brain by Abramson and Weinstein [242] and by Reid [178] who uses lung extract as a source of thromboplastin Reid employs a mechanical shaker for the plasma to increase the accuracy of the end point of the coagulation time Owren [182] has modified the one stage method by using prothrombin free ox plasma to supply a surplus of factor V (p 692) in the coagulation mixture

Shapiro [325] and Brambel and Loker [326] have shown that greater

sensitivity is obtained in the Quick method if the plasma is diluted 12.5 per cent. Rosenfield and Tuft [79] use plasma to which barium sulphate is added as a diluent and standardize the thromboplastin by determining prothrombin times on a plasma pool from at least five normal subjects. Trommeyer [217] uses lyophilized prothrombin free human or bovine plasma stored at − 20° C for ten to twenty weeks as a diluent, the material being adjusted to pH 7.3 before use.

A method utilizing a drop of blood in a loop coagulometer has been devised by Uhn and Barrows [328]. On rotating the loop the drop of blood moves readily on the wire, the clotting point is indicated by cessation of this movement.

Control determinations of prothrombin time are essential as Page and De Beer [329] have shown that periodic fluctuations may occur in the same individual over a period of a few weeks.

Using the Quick technique and assuming twelve seconds for a normal plasma prothrombin time prothrombin activity can be expressed as a percentage of normal from the expression:—

$$C = \frac{k}{pt + a}$$

in which C = prothrombin concentration expressed as per cent. of normal, pt = prothrombin time, and k and a are constants with a value of 303 and 8.7 respectively, provided twelve seconds is the value for normal plasma.

Blood and Serum Coagulation Times. Smith, Ziffren and their co workers use whole blood instead of plasma [42, 76]. This method is suitable for the bedside. 0.1 ml. of a standard thromboplastin solution prepared from rabbit or beef lung is placed in a small serological tube which is filled with venous blood up to a 1 ml. mark. The contents of the tube are mixed and the tube tilted every second or two to observe when clotting occurs. Clotting activity (per cent. of normal) =

$$\frac{\text{Clotting time of normal control}}{\text{Clotting time of patient}} \times 100$$

A modification of the Smith method has been made by Huber and Shrader [194] as a simple method for examining the coagulation time in the newborn. Micro methods have also been elaborated to overcome the objection of venipuncture [74, 75, 155, 162, 327, 398].

Schwager and Jaques [78] determine the prothrombin time of whole blood by adding the latter to thromboplastin at the time the blood sample is drawn. The thromboplastin, prepared from desiccated rabbit brain according to Quick's method, is dispensed in capillary tubes and stored frozen, so that it is available for each determination. The blood is drawn with syringe needle and glassware treated with silicone, which avoids use of an anticoagulant.

Fullerton and Anastasopoulos [84] have emphasized the necessity of a lipoid factor in the coagulation of blood. They obtain values of from 9.3 seconds to 25.4 seconds for normal prothrombin times using various methods with and without lecithin, which is a lipoid.

Thrombokinase	*Prothrombin Time*
Venom	22.5 secs.
Brain powder	24.1 "
Venom + lecithin	9.3 "
Brain powder + lecithin	25.4 "
Brain powder + venom	10.8
Brain powder + venom + lecithin	10.2

They point out that since optimal concentrations of thrombokinase and the lipoid factor are assured using venom and lecithin to determine the pro

thrombin time it would seem preferable to the method using only brain extract, the lipoid content of which is variable Fullerton and Anastasopoulos obtained accelerated clotting times using the venom method if the test subject consumed a meal rich in fat before the test was carried out

They have also shown that slight hæmolysis produces considerable shortening of the accelerated clotting time when the venom method is used, but not with the venom and lecithin and brain extract tests Hence when the venom method is used the greatest possible precautions must be taken against hæmolysis, e g, the syringe used for withdrawing blood must be dry and oxalate solution, not crystals, must be used as an anticoagulant It does not matter what technique is employed in a given laboratory provided conditions are scrupulously standardized and repeated for each [illegible]

Because of the differences in potency of [illegible] preparations employed and [illegible] time is not a satisfact[illegible] various thromboplastin [illegible] thrombin values The various workers using [illegible] and various sources of thromboplastin Thus give normal values of from eleven to thirty seconds Preferably the prothrombin level of an unknown plasma is expressed in terms of the percentage of normal concentration as derived from a prothrombin dilution curve This curve, which is hyperbolic should be redrawn for each lot of thromboplastin employed The rate of formation of thrombin in plasma after the addition of thromboplastin is accelerated or retarded by factors (*e g*, Quick's labile factor, Ware's globulin factor and Owen's factor V) other than prothrombin itself Stefanini [402] has devised a new one stage procedure for the estimation of both prothrombin and labile factor in plasma

Although the one stage method is not as accurate as the two stage for estimating prothrombin, it gives a better estimate of the chance of bleeding in a patient Allen and Vermeulen [197] have pointed out that not only the prothrombin level, but the vitamin K reserves of the body are important in assessing a hæmorrhagic tendency Unfortunately there is no method of measuring the vitamin K reserve Apparently a patient may be at the point of depleting his reserve and still have a normal plasma prothrombin level

Prothrombin Determination. Two-Stage Method

This method, originally devised by Warner, Brinkhouse and Smith [66], is accurate but difficult to perform It is a more accurate measure of the available prothrombin but the one stage method is a more accurate gauge of the likelihood of bleeding in a patient [397] In the first stage the prothrombin of the blood is converted to thrombin with an optimal amount of calcium and an excess of thromboplastic substance In the second, or clotting stage the amount of thrombin formed is measured by the time required for the clotting of a standard fibrinogen solution It is assumed that the amount of thrombin is a measure of the amount of prothrombin originally present and that the clotting time depends upon the amount of thrombin One unit of thrombin is defined as that amount which under specified conditions makes 1 ml of a fibrinogen solution clot in fifteen seconds at 28° C One unit of thrombin is formed from one unit of prothrombin Normal human blood plasma contains approximately 300 units of prothrombin per cubic millilitre The manipulations in the two stage method ensure conversion of inactive component A (p 691) to the free form, so that this method estimates total component A

The two stage technique is carried out by defibrinating oxalated plasma, which is then serially diluted and incubated with calcium chloride, a preparation of thromboplastin and acacia After this a standard fibrinogen solution is added and the clotting time determined The dilution which will give a final concentration of 1 unit of prothrombin per cubic millilitre is determined, and the dilution is then an exact measure of the number of prothrombin

units of the plasma Thus normal human plasma must be diluted 300 times before it contains 1 unit of prothrombin per cubic millilitre If a plasma contained 100 units of prothrombin units per cubic millilitre, the prothrombin concentration would be 33 3 per cent. of normal The two stage method is the one of choice in anticoagulant therapy.

The two stage method has been improved by Stewart and Rourke [67], and Herbert [154] Ware and Seegers [265] have shown that prothrombin conversion is both retarded and incomplete when accelerator globulin is decreased or absent, and thus may occur under certain pathological conditions in which hypoprothrombinæmia has been observed. Ware and Seegers [[illegible]7] have accordingly modified the two stage method by add[illegible] of accelerator globulin Other factors hav[illegible] accelerate the conversion of [illegible]

Shinowar[illegible] by an [illegible] in the prothro[illegible]ivated with calcium and pl[illegible]ated with a fibrinogen solution Sternbe[illegible] its prothrombin by removing fibrinogen from oxalated plasma with thrombin, which is inactivated after standing ten minutes by the antithrombin present in blood The antithrombin activity is then suppressed with alcohol, and the prothrombin converted to thrombin with milk (a source of thromboplastin) and calcium. The amount of prothrombin is estimated by adding the resulting preparation to normal plasma and recording the thrombin fibrinogen clotting times. The method is independent of the activity of the reagents used.

At present it appears to be difficult if not impossible to compare prothrombin levels reported from one laboratory with those obtained in another There is often considerable discrepancy in the two methods, for example in the prothrombin of infants' blood, stored blood, and blood from patients treated with dicoumarol. Mawson [77] has compared the various one-stage techniques with the two stage method and concludes that when plasma from patients treated with dicoumarol is used the two stage method gives results in fair agreement with those obtained by the one-stage method in which Russell viper venom and lecithin are used as the source of thromboplastin When rabbit brain or ox lung is used, the prothrombin concentration is lower than that found by the two stage method

The prothrombin activity of stored or "bank" blood increases during the first four or five days, probably due to disintegration of platelets After the seventh day it falls and reaches forty-eight per cent. of its initial value by the end of three weeks [174] Stored blood a week old is quite suitable for transfusion to correct a lowered prothrombin level. Stored frozen and dried citrate plasma is also an adequate source of prothrombin for transfusion purposes Even after long storage the prothrombin activity is only slightly diminished After fifteen months storage the activity is still seventy to ninety five per cent of normal, and after four to six years it is still almost as active [141] Oxygenation of blood causes prolongation of the prothrombin time, possibly by inactivating the labile factor [388].

CLINICAL MANIFESTATIONS OF HYPOPROTHROMBINÆMIA

A hæmorrhagic tendency due to a reduction of prothrombin only occurs when the level has fallen considerably. Quick [168] puts the critical level at fifteen to twenty per cent of normal (11 to 12 5 secs) Later he stated that a prothrombin time of nineteen to twenty secs indicates a potential hæmorrhagic state [372] Kark and Souter term the hæmorrhagic condition resulting from marked hypoprothrombinæmia hæmorrhagic hypoprothrombinæmia, and divide it into two classes, latent and spontaneous [170]

Latent hæmorrhagic hypoprothrombinæmia occurs at the sites of obvious

trauma when the prothrombin level has fallen to about thirty five per cent of normal Operation wounds begin to ooze or bleed, and the gums bleed if the teeth are vigorously brushed Hæmatomata appear if the skin is pricked or a vein is punctured

A spontaneous hæmorrhagic diathesis appears according to Kark when the blood prothrombin has fallen to fifteen to twenty per cent of normal, and is seen particularly in the newborn, in idiopathic steatorrhœa, obstructive jaundice and severe parenchymatous hepatic disease Large hæmatomata may appear on the back, thighs and other pressure areas, and hæmarthrosis, hæmatemesis, epistaxis, hæmaturia or melæna may occur Menorrhagia, retinal hæmorrhage and in the case of infants intracranial hæmorrhage have been noted Intractable hæmorrhage from trivial wounds and in infants umbilical hæmorrhage have been described Bleeding may manifest itself by a slow oozing from the gums, nose and post operatively from wounds The blood coagulation time is prolonged but capillary fragility is unaltered

VITAMIN K THERAPY

The need for treatment with vitamin K depends on the clinical detection of vitamin K deficiency or on the estimation of the prothrombin time

Vitamin K concentrates obtained from alfalfa or from cereal grass were originally us

to 50 000)

given at the

have been replaced by synthetic analogues which are all derivatives of 2 methyl 1 4 naphthoquinone (menaphthone, menadione) The dosage employed is 1 to 10 mg orally or intramuscularly, although 2 mg daily by mouth or every few days parenterally is probably effective in most cases [223, 232, 331] In the case of infants a dose of 1 mg is sufficient Menaphthone is oil soluble and is therefore injected in oil intramuscularly It can be prepared in a form suitable for intravenous use by dissolving in alcohol and diluting with ten per cent glucose solution [230], although this is unnecessary since water soluble derivatives such as the phosphate and bisulphate of 2 methyl 1 4 naphthohydroquinone and menadoxime are available (p 688) An intravenous injection of one of these will control hæmorrhage due to hypoprothrombinæmia in one and a half to three hours and produce a normal prothrombin level in twenty four to forty eight hours [169] Treatment must be maintained by intramuscular injections of the oil or water soluble compounds or by means of oral preparations Kark and

Compound	Solubility	Dosage	Route of Administration
Vitamin K_1 Oxide	Insoluble in water	10 mg daily	Intramuscular or intra venous (Solubilized by dissolving 10 mg in 3 ml alcohol and diluting with 10 ml saline)
Menaphthone (Menadione)	Oil soluble	1–5 mg daily	Oral or intramuscular
Acetomenaphthone	Oil soluble	2–10 mg daily	Oral
Menaphthone bisulphite (2 methyl 1 4 naphthohydro quinone 3 sodium sulphon ate Hykinone)	Water soluble	5 mg every 12 hours	Oral
		2 5 5 mg daily	Subcutaneous, intra muscular or intravenous
4 Amino 2 methyl naphthol hydrochloride (Synkamin)	Water soluble	4 mg every 12 hours	Oral
		1 5 mg daily	Subcutaneous intra muscular or intravenous
Tetrasodium 2 methyl 1 4 naphthohydroquinone diphosphoric acid ester (Synkavite)	Water soluble	5 mg every 12 hours	Oral
		5–10 mg	Subcutaneous intra muscular or intravenous

Souter [169] have shown that bile salts are not necessary for the absorption of vitamin K analogues by mouth although it is claimed that they increase their effectiveness [350] The following is a list of synthetic vitamin K preparations that have been used with their dosage and route of administration If menaphthone or the water soluble analogues fail, the more potent vitamin K_1 oxide should be tried [429]

The treatment of hypoprothrombinæmia with just sufficient vitamin K to raise the prothrombin level of the blood to normal will not ensure against hæmorrhage except for very short periods Treatment must be continued beyond this point to build up a reserve in the body before operation and the administration of the vitamin continued post operatively Unless this is done the prothrombin level will fall when treatment is withdrawn, with danger of subsequent hæmorrhage In hæmorrhage due to hypoprothrombinæmia a blood transfusion is the first necessity not vitamin K therapy which can be given later The danger of post operative bleeding is not passed until the tenth day after operation In patients with severe hepatic damage hypoprothrombinæmia cannot be corrected even with large amounts of vitamin K Blood transfusions from donors given large doses of vitamin K are also ineffective in such cases [14]

There appears to be no danger from overdosage with vitamin K or its analogues Unger and Shapiro [381] have induced hyperprothrombinæmia in normal subjects with very large doses of a water soluble analogue but it was only transitory and lasted twenty four to forty eight hours Stewart [232] records only three cases in which the plasma prothrombin was raised to more than 110 per cent of normal by vitamin K, one patient was dehydrated and the other two suffered from recurrent thromboses of peripheral veins He has given up to ten times the effective dose of vitamin K without untoward effects and without elevating the plasma prothrombin above normal

Hyperprothrombinæmia has been recorded in untreated acute thrombophlebitis in the initial stage of embolism, and in multiple myeloma [330] and in the late night hours and early morning in arteriosclerotic patients [60]

CLINICAL USES OF VITAMIN K

In Conditions not Associated with Jaundice or Liver Disease Vitamin K cannot be used to check bleeding irrespective of its origin nor has it any appreciable effect on the prothrombin level of subjects not suffering from hypoprothrombinæmia [267] It is not of value in hæmophilia purpura, and diseases of the blood forming organs, although there is a tendency to bleed in these conditions there is no deficiency of prothrombin Nor is the vitamin effective for the control of hæmorrhage in the normal individual

It is now being realized that prothrombin deficiency may be encountered in other conditions particularly those associated with an abnormal state of the gastro intestinal mucosa, which interfere with the absorption of the vitamin Patients with gastro intestinal disease also consume diets poor in vitamins Thus Snell and his co workers [82, 88 138] have reported occasional instances of vitamin K deficiency in ulcerative colitis polyposis of the colon regional ileitis, pyloric obstruction, sprue and gastro jejunocolic fistula A hæmorrhagic diathesis in sprue is not common, but it has been reported by several observers [169 176] Bercovitz and Page [310] have shown that some thirty per cent of patients with ulcerative colitis have a lowered prothrombin level and that the administration of vitamin K analogues causes a marked diminution in the mucous membrane bleeding seen in this condition

The plasma prothrombin levels of thirteen patients suffering from intestinal lesions were examined by Butt, Snell and their co workers [82 94] The cases included ulceration, external and internal fistulæ, intestinal obstruction diarrhœa and collapse of the ileum A marked hypoprothrombinæmia was observed in many of the patients, and in three hæmorrhage

occurred The vitamin K deficiency in these patients was considered to be due to an insufficient amount of normal intestinal mucosa for adequate absorption. Butt and Snell suggest that the blood prothrombin should be determined as a prelude to therapy if bleeding occurs in any medical or surgical case in which a diminution of the absorptive surface of the intestine is probable or suspected, or in which biliary obstruction or infection is present. Although there may not be spontaneous hæmorrhage before, if patients with a low prothrombin level are submitted to surgical procedures the hæmorrhage may become serious

Others have recorded low prothrombin levels in severe diarrhœa, intestinal obstruction, fistulæ, mesenteric obstruction, hæmorrhagic retinitis, and in pseudo hæmophilia hepatica of childhood [89] Stewart [196] and his colleagues have reported prothrombin deficiency in patients with peptic ulcer, malnutrition and cachexia According to Warner and Owen [264], patients with pernicious anæmia show prothrombin levels of only forty to sixty per cent of normal, the hypoprothrombinæmia is not rectified by vitamin K therapy unless liver is given simultaneously

Moderate reduction in the prothrombin level in such diverse conditions as duodenal ulcer, melæna, lung abscess and rectal carcinoma has been observed by Stewart and Rourke [96] Treatment with vitamin K and cholic acid resulted in a gradual rise in the prothrombin level Rawls [234] has also observed a hypoprothrombinæmia in patients suffering from rheumatoid arthritis, hepatitis due to cincophen, hæmorrhage from gastric ulcer, myelogenous leukæmia, aplastic anæmia, thrombocytopenia, hyperthyroidism, malignant endocarditis, chronic intestinal diseases, and toxic reactions due to the administration of gold In most of these the prothrombin level returned to normal after administering vitamin K Thordarson [335] noted a low prothrombin level in ten out of fourteen patients with myeloid leukæmia The administration of vitamin K_1, however, failed to raise it to normal

It has been claimed that vitamin K is effective in the treatment of menorrhagia [336] although another worker investigating three hundred gynæcological cases found the prothrombin time within normal limits [91]

Low prothrombin levels are present in Banti s syndrome and in the early stages only is vitamin K effective in restoring prothrombin [257] It is recommended as part of the treatment in preparation for splenectomy.

Vitamin K has failed to control pulmonary hæmorrhage (pp 698, 714)

In the treatment of hypoprothrombinæmia resulting from intestinal disorders vitamin K analogues should be given parenterally as they may not be effectively absorbed by mouth Five to ten mg of a water-soluble analogue is given parenterally daily or 1 mg to 2 mg menaphthone in oil intramuscularly Hepatic function if impaired should be treated and blood transfusions given if necessary

In Jaundice and Liver Disease. *Incidence* The hæmorrhagic tendency in patients suffering from obstructive jaundice and diseases of the liver has long been known, and constitutes a distinct hazard in the case of patients submitted to operation Thus in nearly four thousand operations on jaundiced patients sixty one of the four hundred and forty-two postoperative deaths (13 8 per cent) were attributed to hæmorrhage [104], a figure which agrees closely with the twelve per cent given by Sir John Fraser [105] A much higher figure is given by Butt [82] Before it was realized that such hæmorrhage was due to a vitamin K deficiency, conditioned by lack or escape of bile salts, the patient was prepared for operation by giving large doses of glucose and by calcium therapy. A review of the older literature reveals that the incidence of bleeding in patients with obstructive jaundice was higher before the development of modern pre operative and post operative care Absence of bile salts in the intestine may occur without obstruction of the common bile duct, as it has repeatedly been shown that bile from the liver following obstruction or severe liver

damage may contain no bile salts All patients with obstructive jaundice should have pre operative prothrombin determinations and operation deferred if possible until the prothrombin level is raised to normal The value of vitamin D in preventing hæmorrhage in jaundiced patients is discussed on p 576

Causes Suggested causes for this considerable fall in the prothrombin level are (1) increased utilization of prothrombin for the formation of exudates, (2) defective absorption of vitamin K due to deficient secretion of bile salts, (3) liver damage resulting from anæsthesia (p 697), infection and operative trauma, (4) lack of adequate vitamin K reserves

Prothrombin Level Bleeding may occur when the prothrombin level falls below thirty to forty per cent of normal [80], values below this may be dangerous No sharp prothrombin level can be considered a bleeding level as some patients with a plasma prothrombin as low as twenty per cent of normal may show no evidence of hæmorrhage Other factors such as trauma infection, operative hæmorrhage, vitamin K reserves, and exudate formation play a part in determining the hæmorrhage level The danger of post operative bleeding in patients suffering from diseases of the liver or biliary passages may persist for as long as ten days after operation, massive and fatal hæmorrhage may occur in a patient with severe liver damage The lowest level is reached between the first and fourth days after operation [106]

It has been suggested that anæsthetics might contribute to the post operative hypoprothrombinæmia in patients with jaundice or disease of the biliary tract Although chloroform produces a hypoprothrombinæmia, Allen and Livingstone [200] have concluded from prothrombin studies on a hundred and six patients who underwent operations under ether, vinesthene, nitrous oxide, ethylene, bromethol (avertin), nupercaine and spinal and local anæsthesia, that these anæsthetics have no effect on the prothrombin level They suggest that some form of storage of vitamin K or prothrombin occurs in the body and that failure to replenish this store in the patient with obstructive jaundice or biliary fistula accounts for the post operative hypoprothrombinæmia

For many years it has been noted that there is liver impairment in hyperthyroidism Andrus and Lord [223] have shown that there is an immediate post operative fall in the plasma prothrombin level, averaging fifteen per cent and amounting to forty per cent or more in some cases The values were similar to those obtained after chloroform anæsthesia (p 693) It would, therefore, seem advisable to improve the hepatic function of the hyperthyroid patient pre- and post operatively

Treatment Despite the fact that patients with liver disease may not appear to need vitamin K as shown by their prothrombin levels, it should be given to all such patients as a routine, particularly if surgery is contemplated whatever the prothrombin level or the presence or absence of bleeding [107] A normal prothrombin on the day of operation is no guarantee that post operative hæmorrhage will not occur Where possible, the dosage of vitamin K should be controlled by repeated prothrombin estimations In conditions in which there is damage to the hepatic parenchyma as in cirrhosis of the liver, acute yellow atrophy, Wilson's disease (hepatico lenticular degeneration), multiple liver abscesses, fatty infiltration of the liver, acute hepatitis and carcinoma of the liver, neither the administration of vitamin K, nor blood transfusions will raise the prothrombin level [107, 144, 314]

Pre operatively 2 to 5 mg of menaphthone or 5 to 10 mg of a water soluble derivative is given orally every day for at least four days or until the prothrombin approaches 70 per cent of normal In the presence of jaundice it is advisable to give bile salts with menaphthone These preparations may be given parenterally if the patient is vomiting or defective absorption is suspected The dosage of menaphthone is 2 mg in oil intramuscularly,

that of the water soluble analogues 5 to 10 mg by any parenteral route

Treatment should be continued regardless of the post operative prothrombin level for two weeks and as long as there is any evidence of liver damage, obstructive jaundice or biliary fistula Therapy should be controlled by frequent prothrombin estimations These should be done daily for four days and then every other day If bleeding occurs or surgical interference is considered 10 to 25 mg of a water soluble vitamin K analogue should be given intravenously as well as whole blood which will provide additional prothrombin for about eight hours Stored or bank blood is only satis factory if it is used within a week of its withdrawal from the donor [174] The prothrombin level of stored blood slowly falls after this time (p 702) The administration of vitamin K to the donor before bleeding has not been found to be of value if the recipient is suffering from severe hepatic damage [14]

Liver biopsy is not a safe procedure unless the prothrombin level is within seventy per cent of normal If it is below this 10 mg of a water soluble vitamin K analogue should be given daily intravenously and continued for forty eight hours after the biopsy

Liver Function Test using Vitamin K Attempts have been made to use the prothrombin response to vitamin K as a test of liver function since hypoprothrombinæmia associated with severe liver damage does not respond to treatment with vitamin K [186 198 266]

Lord and Andrus [223] and Allen and Julian [337] have suggested that the response of plasma prothrombin to intramuscular injections of 2 methyl 1 4 naphthoquinone may be used to differentiate between intrahepatic and extrahepatic jaundice Lord and Andrus found that there was a rise of from ten to sixty two per cent in the plasma prothrombin following the intra muscular injection of 2 methyl 1 4 naphthoquinone in extrahepatic jaundice (common duct stone cholangitis carcinoma of the head of the pancreas stricture of the common duct) whereas in cases of intrahepatic jaundice the response was ten per cent or less The cases of extrahepatic jaundice were confirmed at operation or autopsy Kark and Souter [224] also observe that in patients with intense jaundice the return of a low prothrombin level to normal after vitamin K therapy favours a diagnosis of obstructive or extra hepatic jaundice They recognize five types of response to vitamin K therapy in patients with liver disease (1) Rapid response in obstructive jaundice (2) no response in gross liver disease (3) partial response with the level still slightly subnormal after vitamin K therapy in cases of acute or subacute parenchymatous hepatic damage of a moderate degree (4) a gradual rise in the prothrombin level with treatment coincident with clinical improve ment in cases of infective cholangitis infectious hepatitis acute or toxic hepatosis and obstructive jaundice complicated by infection (5) a fluctuating prothrombin level above the threshold for hæmorrhage in patients with chronic and long standing hepatic disease usually unassociated with jaundice

Begtrup and Hansen [83] found that the response to vitamin K confirmed the diagnosis of extrahepatic jaundice in seventy seven per cent of one hundred and fifty two cases and of intrahepatic in eighty six per cent The corresponding figures for the Takata Ara test were sixty five per cent and sixty per cent respectively for the urobilin excretion test seventy nine per cent and thirty two per cent and for the galactose tolerance test fifty five per cent and fifty eight per cent In chronic liver impairment the figures for the various tests were eighty five per cent seventy seven per cent thirty three per cent and twenty two per cent Unger and Shapiro [338] have shown that the response to vitamin K gives excellent correlation with other liver function tests and with the clinical findings

Owren [272] has shown that in intrahepatic jaundice a concentration of factor V (p 692) under fifty per cent of the normal value carries a bad prognosis A steadily falling factor V concentration indicates malignancy

with a fatal outcome while persistently subnormal values suggest a chronic hepatitis According to Owren factor V may be normal or increased in *obstructive jaundice*

Allen [384] in a critical review of liver function tests states that the prothrombin res K
excretion test or
In the jaundiced
intrahepatic jaundice the character of the prothrombin response to vitamin K may n all other methods short of surgery have conditions for performing the pro thro a significant prothrombin reduction *(less than seventy five per cent)* *the patient must be eating well and afebrile* a sensitive prothrombin method should be used and the vitamin K must be given parenterally as a water soluble analogue in two doses of 10 mg several hours apart In obstructive jaundice there is at least a twenty five per cent increase in the prothrombin whereas in cases of extensive liver damage there is little or no response These observations have been confirmed by Stein [383] who claims to have achieved an accuracy of over ninety five per cent in differentiating between intra and extrahepatic jaundice using the prothrombin response to vitamin K test Of course the interpretation *of the test may be difficult in cases of combined intra and extra hepatic* jaundice *e g* carcinoma of the liver with metastases in the portal glands and biliary cirrhosis following chronic obstruction of the bile ducts

A summary of the results of various workers using the response to vitamin K t
of hepatic dis success to differentiate extrahepatic from intrahepatic jaundice The usefulness of the test is limited by the fact that jaundice is not always accompanied by *prothrombin deficiency* *The test is of no prognostic value as it does not* differentiate between obstruction due to neoplasms and other lesions

Hypoprothrombinæmia in the Newborn It was originally observed by Brinkhous [111] and it has been repeatedly confirmed since that the prothrombin level of the newborn and in early infancy is lower than normal Values from ten to forty per cent of normal have been recorded [111 120] According to Waddell and his co workers [113] the period of most marked deficiency occurs from forty eight to seventy two hours after birth the prothrombin level returns to normal at the beginning of the second week [194] It is of interest that according to the Mosaic law circumcision was delayed until the eighth day because of the risk of bleeding if done earlier If vitamin K or an analogue is administered within twenty four hours of birth this fall in prothrombin is prevented [203] It is also prevented if the mother is given an intramuscular injection of vitamin K or an analogue shortly before delivery which shows that vitamin K must pass through the placenta. Normally the cord blood prothrombin is much lower than that of the maternal blood indicating that natural vitamin K does not pass readily from the mother s blood through the placenta

It has been suggested that the hypoprothrombinæmia of the newborn is due to a low food intake just after birth and to the low vitamin K content of milk The gut of the newborn is also sterile so that bacterial synthesis of vitamin K which normally occurs in the gut does not take place Salmonsen and Nygaard [121] observed that supplementary feeds of cows milk—which was *probably contaminated with vitamin K producing organisms*—helped to prevent the fall in the prothrombin level after birth Cellis and Lyon [949] also noted that supplements of evaporated milk and corn syrup which form a good bacterial medium had a similar effect Macpherson [340] believes that the prothrombin level in the newborn is dependent on the mother s diet although this has been denied by others [159 203 344] In his series the

PROTHROMBIN RESPONSE TO VITAMIN K TEST

Author	Method of Estimating Prothrombin	Technique of Test	Interpretation (24 hours later)	Accuracy of Test
Begtrup and Hansen [83]	Thordarson Begtrup and Hansen's method [83]	2 mg of 2 methyl 1 4 naphthohydroquinone disuccinate by mouth	Increase of 30% or more indicates obstructive jaundice less than 30% suggests parenchymatous jaundice	77% 86%
Lord and Andrus [223]	Two stage (p 701), expressed as percentage of normal prothrombin concentration	2 mg of menaphthone i m	Increase of 10% suggests extrahepatic jaundice, less than 10% intrahepatic Repeat 48–72 hours later less than 15% rise indicates intrahepatic jaundice	94%
Allen [384]	Modified Quick serial dilutions expressed as percentage of normal	Two 10 mg doses of water soluble preparation i v several hours apart	Original level less than 75% Rise to normal in 24 hours in obstructive jaundice Response of less than 25% indicates intrahepatic jaundice	99%
Stein [383]	Modified Quick expressed as percentage of normal	5 10 mg menaphthone i m	Original level less than 75% If rise exceeds 10% dose repeated In obstructive jaundice returns to more than 85% after 5 10 mg If less than 85% indicates intrahepatic jaundice	95%
Althausen [97]	Quick expressed as percentage of normal	1 mg menadione bisulphite parenterally	Original level 70% or less If increase 20% or more indicates obstructive jaundice	94%
Unger and Shapiro [338]	Modified Quick using dilution to 12 5% (normal 39 5 sec ± 2 5 sec)	76 mg Synkayvite i v daily for 3 days	Failure to return to normal prothrombin time by third day indicates impaired liver function	100% in cases confirmed by biopsy 91% in cases confirmed clinically

prothrombin levels of the blood of newborn infants of *adequately fed mothers* was considerably higher than those of infants whose mothers subsisted on inadequate diets Some of the children of the poorly fed mothers suffered from hæmorrhagic disease and three showed *signs of cerebral irritation* which was attributed to cerebral hæmorrhage resulting from a lowered prothrombin level Macpherson also considers that prolonged labour and the use of anæsthetics causes a fall in the prothrombin level of *infants* blood sufficient to cause hæmorrhage It is not certain whether the anæsthetics and barbiturates used in labo[illegible]
According to Fitzgerald and
barbital and sodium amyl br
produce not only a lowered prothrombin level in the infant but increase hæmorrhage They state that this can be prevented by administering

FIG [illegible]

vitamin K to the mother in labour These views have been challenged [299]

The work of the Randalls [195] suggests that more than a deficiency of prothrombin is involved in the apparent low prothrombin levels of the new born as measured by standard methods because the plasma of infants normal by the one stage test is less effective than adult plasma in restoring the prothrombin level of deficient infants According to Quick [270] newborn infants plasma is deficient in component A (p 691) If this were so the plasma of patients receiving dicoumarol or suffering from liver disease should restore the prothrombin time of the plasma of these infants but this is not so according to the Randalls They also state that Quick s labile factor (p 691) does not seem to be the basis of the prothrombin deficiency of the newborn because stored plasma in which this factor is absent or diminished is as effective as fresh plasma for restoring prothrombin times Perhaps most significant is their finding that stored serum from which prothrombin thrombin and fibrinogen would have disappeared is also effective in bringing the prothrombin times of newborn infants to normal It would appear from

the work of the Randalls that the prolonged prothrombin times seen in the newborn as measured by the one stage method cannot be adequately explained in terms of a deficiency of prothrombin itself or of any of the classical clotting factors A possible explanation is that the newborn infant is unable to convert prothrombin to thrombin with the same speed and efficiency as the normal adult

Hæmorrhagic Disease of the Newborn Clinical This is characterized clinically by hæmorrhage into the gastro intestinal tract (melæna hæmatemesis) bleeding from the cord (omphalorrhagia) nose, palate the genito

FIG 245 Delayed hæmorrhagic disease of the newborn with intracranial hæmorrhage superimposed on a previous interventricular hæmorrhage The infant who shows marked opisthotonus also had severe diarrhœa and oral hæmorrhage Subsequent therapy with vitamin K and a blood transfusion failed to save the infant's life Autopsy revealed petechial hæmorrhage in the brain and massive hæmorrhage into the ventricles The diarrhœa undoubtedly contributed to the hæmorrhagic state and depressed the prothrombin level so low that a poorly formed clot at the site of a previous hæmorrhage was dislodged and further hæmorrhage resulted It is probable that hæmorrhage into the ventricles had continued since birth and that when they were distended opisthotonus developed

urinary tract vulva and the suprarenals The commonest first symptom is the passage of a large tarry stool but the condition may be ushered in by hæmatemesis bleeding from the umbilicus (Fig 244) pallor due to internal hæmorrhage or the rapid enlargement of a cephalhæmatoma The child may pass enormous quantities of blood in the stools and if the hæmorrhage is not arrested it may die on the fourth or fifth day In the differential diagnosis of hæmorrhage of the newborn the following must be considered birth trauma infection and sepsis congenital thrombopenia constitutional fibrinopenia hereditary hæmophilia and pseudo hæmophilia The incidence of hæmorrhage of the newborn is stated by various authorities to be from 1 in 150 to 1 in 500 births [122 361]

Although it is generally considered that hæmorrhagic disease of the newborn occurs between the second and sixth days after birth it has been stated that in a third of one series studied it occurred on the first day [118] Neonatal hæmorrhage *in utero* has also been reported by Javert [119] who suggests that uterine contractions increase the intracapillary pressure of the fœtus and tend to produce hæmorrhage which becomes pathological when the clotting mechanism is disturbed

Intracranial Hæmorrhage It is believed by some workers that intracranial hæmorrhage in the new born is associated with hypoprothrombinæmia and that obstetric trauma may only play a secondary *rôle* [113 115 122 124] It is conceded that the trauma of birth may in many instances cause the appearance of small bleeding points in the intracranial cavity However even in normal births and in babies born by cæsarean section such bleeding points have been demonstrated and death from cerebral hæmorrhage has been reported after cæsarean section [204] With an abnormal clotting mechanism such as will result from a temporary hypoprothrombinæmia no clot will form and slow oozing will continue With a normal clotting mechanism bleeding from small vessels would cease This slow oozing from the bleeding points might explain why the symptoms of intracranial hæmorrhage often only reveal themselves on the fourth to sixth day after birth (Fig 245) Thus out of sixteen cases of intracranial hæmorrhage at the Children s Hospital Washington D C the average date of onset was 6 3 days after birth [125]

Treatment of Hæmorrhage of the Newborn The general consensus of opinion is that hæmorrhage of the newborn is due to hypoprothrombinæmia and that the administration of vitamin K or one of its analogues restores the prothrombin to normal levels and controls the hæmorrhage [112 116 124 127 130] From a study
prophylactic administrat
the mort
[255] of ... a neonatal mortality
of 1 9 p ... received vitamin K
prophylactically during labour, the mortality among a comparable control group whose mothers did not receive vitamin K was 3 9 per cent Statistical analysis
These
and by
born infants were unable to find any correlation between the prothrombin level and the frequency of what they term hæmorrhagic manifestations These occurred in 6 59 per cent of 711 newborn infants given vitamin K and in 6 6 per cent of 982 untreated controls The mortality was the same in the two groups Parks and Sweet [351] and Hay Hudson and Rodgers [410] were also unable to observe any reduction in the incidence of neonatal hæmorrhage by administering vitamin K to the mother before birth These conclusions have been criticized by Waddell [251] Hay and his co workers [410] observed hæmorrhagic disease of the newborn in 0 24 per cent of 4 602 births after giving the mother vitamin K before birth and in 0 19 per cent of 12 131 control births The difference is not statistically significant Potter states that there is no real proof that prolongation of the prothrombin time is a direct cause of neonatal hæmorrhage She found that many infants with an excessively prolonged prothrombin time show no evidence of hæmorrhage while others with relatively little prolongation bleed severely According to her true hæmorrhagic disease is exceptionally rare the cause of most neonatal hæmorrhage being trauma Potter bases her views on a study of 6 560 infants whose mothers received vitamin K before birth and 6 630 untreated controls The fœtal mortality in the two groups was 29 8 and 25 8 respectively Both Sanford and Potter state that evidence of bleeding is not a justification for the diagnosis of true hæmorrhagic disease

in which there is a prolongation of the prothrombin time and vitamin K is of value. They believe that conditions other than prolonged prothrombin time and vitamin K deficiency are responsible for the majority of hæmorrhagic manifestations in the newborn. The explanation of these anomalous results depends upon the interpretation of the expressions "hæmorrhagic manifestation" and "hæmorrhage of the newborn." In a small group of premature infants it was observed that there were just as many hæmorrhagic manifestations in premature infants given vitamin K as in untreated controls, and the mortality and frequency of cerebral hæmorrhage as shown by necropsy findings was actually higher in the treated group [394].

A voluminous literature that is unnecessary to detail has arisen on the prophylactic treatment of the hypoprothrombinæmia of the newborn with vitamin K analogues. Shettles and his colleagues [129, 255], at Johns Hopkins Hospital, were the first to give vitamin K to the mother before birth. They gave it daily for several weeks before delivery; it is now known that a single dose a few hours before delivery is sufficient to produce a normal prothrombin in the infant after birth. A dose of 20 mg by mouth given to the mother early in labour or 10 mg of a water soluble analogue parenterally late in labour prevents hypoprothrombinæmia in the newborn. If the infant is given 1 mg of a vitamin K preparation intramuscularly daily for the first few days after birth the prothrombin is restored to normal adult levels. Should, however, the infant be bleeding a blood transfusion is essential and should be given in addition to vitamin K [348, 352].

It has been stated that healthy newborn infants do not need vitamin K as their prothrombin level returns to normal spontaneously and that it should only be given to those with birth injuries or in cases of hæmorrhage in addition to blood transfusion [333].

Gastro-Intestinal Diseases of Infancy and Childhood. Diseases characterized by chronic or subchronic gastro-intestinal lesions often develop hypoprothrombinæmia with or without bleeding. The literature contains reports on post operative hæmorrhage in the pyloric stenosis of infants after Ramstedt's operation [293].

Cases of congenital obstruction of the alimentary tract show hypoprothrombinæmia, partly because no food and hence exogenous vitamin K, is consumed and partly because there is no bacterial synthesis of the vitamin. Since surgery is imperative to save life, the hypoprothrombinæmia should be corrected with vitamin K before any operative procedure. Grossman [258] quotes a case of congenital jejunal obstruction in which hæmorrhage occurring after operation was controlled with vitamin K, and one in which a child with duodenal stenosis was operated upon without vitamin K therapy and died from hæmorrhage.

Cœliac disease in children, like sprue and idiopathic steatorrhœa in adults is sometimes associated with hæmorrhagic manifestations. Fanconi [89] suggested that this was due to vitamin K deficiency caused by malabsorption of fats. Other authors have confirmed the presence of hypoprothrombinæmia in these conditions [95, 170].

Pseudohæmophilia Hepatica and Hereditary Pseudohæmophilia. Pseudohæmophilia hepatica is a rare hæmorrhagic syndrome accompanying acute destruction of liver tissue in infectious and toxic states such as syphilis, acute yellow atrophy, and poisoning by such drugs as arsenic, chloroform and the sulphonamides. The bleeding is due to a decreased production in the liver of prothrombin or fibrinogen or both, with a resultant prolongation of clotting time. Kugelmass [126, 137] describes the treatment of a case of infective jaundice with hæmorrhagic manifestations that responded favourably to the administration of vitamin K. Another case of hæmolytic anæmia, due to the toxic effects of sulphanilamide, also improved when given vitamin K in the same dosage.

Hereditary pseudohæmophilia is a syndrome appearing in both males

and females characterized by a hæmorrhagic tendency, prolonged bleeding time, normal clotting time and a normal platelet count It may be present at birth or latent for many years, the hæmorrhage developing as a result of an inherent qualitative defect in the blood platelets A case of this condition with a low prothrombin level was satisfactorily treated with vitamin K by Kugelmass [126] He is careful to point out, however, that since the hæmorrhage is primarily determined by defects in the platelets, vitamin K therapy is only indicated when there is a coincident decrease in the prothrombin level

Retinal Hæmorrhage There have appeared in the ophthalmological, obstetrical and pediatric literature numerous theories explaining the ætiology of retinal hæmorrhage in the newborn Maumenee, Hellmann and Shettles [163] attribute it to vitamin K deficiency They state that newly born infants with retinal hæmorrhage show lower prothrombin levels than normal infants and they believe that the incidence of the condition is reduced by administering vitamin K to the mother before labour. Two milligrams of menaphthone were given by mouth daily for at least four days before delivery They point out that the vitamin has very little effect if given during labour By giving expectant mothers 2 mg of menaphthone daily for four days before the onset of labour the incidence of retinal hæmorrhage was reduced to four per cent compared with twenty five per cent in controls [255]

Pray and his co workers [259] also report a reduction in the incidence of retinal hæmorrhage in the infants of mothers treated with 10 to 20 mg of menaphthone during or before labour They obtained the best results by treating the mother before the onset of labour Wille [202] stresses that vitamin K must be given to the mother every day in the last months of pregnancy to obtain any reduction in the incidence of retinal hæmorrhage in the newborn

Falls and Jurow [250] were unable to observe any reduction in the incidence of retinal hæmorrhage in the newborn within two days of birth after giving the mother vitamin K during labour or giving it to the infant after delivery They believe that trauma and anoxia are important factors in producing retinal hæmorrhage in the newborn and point out that at birth, when the hæmorrhages can be seen, prothrombin levels are usually normal

This reduction in the incidence of retinal hæmorrhage in newborn infants by means of vitamin K therapy is significant because of the possible relationship between retinal and intracranial hæmorrhage

Effect of Vitamin K on Thrombosis. Morton, Shearburn and Burger [273] have investigated the effect of vitamin K on thrombus formation The post partum incidence of thrombosis was 0 14 per cent in a group of seven hundred pregnant women given vitamin K, the incidence in a group of 5,728 controls studied over a ten-year period, who had not received vitamin K, was 0 48 per cent In animal experiments the leg veins of two groups of dogs, one of which received vitamin K, were traumatized and sections later removed and examined microscopically There was no significant difference in the incidence of thrombosis in the two groups Doles [371] states that in acute coronary occlusion there is a hypoprothrombinæmia and that the prompt administration of 50 to 72 mg of a soluble vitamin K analogue every six to eight hours intravenously or intramuscularly diminishes pain and prevents the formation of thrombi

Hæmoptysis Levy [321], Bauer [237] and Sheely [226] state that the intensity and duration of hæmoptysis in tuberculous patients—who often show low prothrombin levels—is considerably diminished after vitamin K therapy On the other hand Kaplan [354], Harrell and Ray [355] Farber and Miller [320] report that vitamin K is of no value in the control of hæmoptysis

Abortion Moore and his colleagues [353] observed that does fed a diet deficient in vitamin K to produce hypoprothrombinæmia and then mated invariably aborted from the tenth to the fourteenth day, and at autopsy

retroplacental hæmorrhages were seen. They suggest that because hæmorrhage occurred at this site the placenta is unduly susceptible to vitamin K deficiency, since the blood prothrombin level did not fall to critical hæmorrhagic levels. Javert and Stander [346] believe that a deficiency of vitamins C and K is a factor in certain cases of threatened and spontaneous abortion. They observed that in seventy nine patients with threatened, spontaneous or habitual abortion ascorbic acid deficiency was found in sixty nine per cent and hypoprothrombinæmia in seventy two per cent. The patients usually complained of skin ecchymoses, epistaxis, bleeding gums and vaginal bleeding. A clinical study of thirty three patients with threatened or habitual abortion showed that after treatment with vitamins C and K, progesterone, minerals and vitamin E the incidence of abortion was twenty and seven per cent in the two groups, while in controls the abortion rate was one hundred per cent in two groups of forty six. As so ... were given it is difficult to evaluate the pre... ing the incidence of abort...

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Certain drugs such as organic arsenicals, sulphonamides, aspirin and salicylates are known to produce hypoprothrombinæmia (p. 696). It has been recorded that vitamin K therapy can raise a lowered prothrombin level when this is due to sulphonamide [306], aspirin and salicylate therapy [319]. Shapiro [319] has shown that approximately 1 mg of menaphthone will counteract the prothrombinopenic activity of 1 gram of acetylsalicylic acid (aspirin) in patients receiving prolonged therapy with this drug. Hypoprothrombinæmia has been recorded in syphilitic patients given massive doses of organic arsenicals [312], but there is no record of this being corrected with vitamin K.

The clinical significance of the hypoprothrombinæmia following the administration of aspirin has been exaggerated. Livingstone and Neary [109] found practically no change in the prothrombin level after administering 21 grams of aspirin for a week.

Other Uses of Vitamin K

It has been suggested that vitamin K might be of value in the treatment of hypertension [36], as it has been found to be active in lowering the blood pressure in experimental renal hypertension [356], although this has been denied [184]. Black [260] observed a diminished prothrombin level in sixty five per cent of one hundred and fifty six patients with urticaria who had obtained no relief from elimination diets, search for infections and allergens and avoidance of drugs. Sixty per cent obtained relief with menaphthone given 2 mg three times a day before meals. Unfortunately the results were not submitted to statistical analysis.

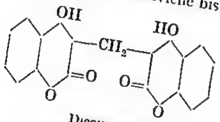
Mitchell [132, 236] observed that the vitamin K analogue tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate produces a small but measureable improvement in the palliative results of X-ray therapy in some cases of advanced cancer. The rationale of the treatment was to block the synthesis of the nucleic acids in the rapidly growing cancer cells, which the above analogue may do as it inhibits mitotic division in chick fibroblast cultures and in some human carcinomata. Many other quinones have an antimitotic action.

VITAMIN K ANTAGONISTS

A number of compounds are known with a composition similar to vitamin K that antagonize its action. When administered to animals on a normal

diet they produce the symptoms of vitamin K deficiency, i e , hypoprothrombinæmia These include 2 3 dichloro 1 4 naphthoquinone [243], 3 hydroxy 1 4 naphthoquinone [261], phenylindandione [279], dihydroxy stearic acid [180] 2 methyl 2 methoxy 4 phenyl 5 oxodihydropyrano benzopyran, also known as "No 63" and cyclocumarol [279, 396, 430, 431] The subject is reviewed by Brown and Douglas [134]

Dicoumarol Dicoumarol, or 3,3′-methylene bis (4 hydroxycoumarin), is



Dicoumarol

a compound present in spoiled sweet clover which produces the condition known as the hæmorrhagic disease of cattle [10] by diminishing the prothrombin level [208] It was first isolated from sweet clover and synthesized in 1941 by Link and his associates [209] of the University of Wisconsin Since then a considerable literature on the pharmacology and clinical applications of this compound has appeared

Mode of Action The oral administration of dicoumarol to animals or human beings results in a reduction of the prothrombin level of the blood, as shown by a prolongation of the prothrombin time [211] There is a latent period of some twenty four to seventy-two hours before the prothrombin time is affected This is because it is slowly absorbed and largely combined with the plasma proteins, with the result that it is slowly eliminated by the kidneys [417] It is largely stored in the liver as shown by tests with dicoumarol containing radio active carbon [407] It has been suggested that dicoumarol inhibits the formation of prothrombin in the liver and that the prothrombin in the blood is reduced before the prothrombin time is prolonged [278] Quick [358] believes that it acts by diminishing or inactivating the B component of the prothrombin complex (p 691), probably by inhibiting an enzyme mechanism which produces prothrombin [427] Other workers believe that dicoumarol not only inhibits prothrombin but another factor associated with the plasma pseudoglobulins [158] Dicoumarol also prolongs [illegible] time and clot retraction time , the bleeding time is [illegible] Dicoumarol decreases the adhesive [illegible] part in blood coagulat[illegible] concentration [illegible] prothrombin ti[illegible] thrombosis is [illegible] level of dicoum[illegible] which must be reached [illegible] any subject there is a threshold plasma [illegible] induced intravascular [illegible] n the [illegible] before a detectable prothrombin response is elicited , this varies from person to person, but is generally between 5 and 10 mg per litre [417]

Pharmacology The principal toxic effects produced by excessive dosage are pulmonary œdema [281] and bleeding, which may occur from the nose, stomach lung bowel, kidney, bladder, skin or beneath the conjunctiva The hæmorrhage is both gross and microscopic In addition there is acute glomerular swelling, a toxic lymphoid tissue reaction [282], rapid respiration, general vasodilatation irregular heart beat, congestion of the liver, and after death rapid rigor mortis [283] If given in adequate dosage vitamin K, and its oxide but not vitamin K analogues, can inhibit the action of dicoumarol although there is a long latent period of about five days before the hypoprothrombinæmia is corrected [256, 284] This effect of vitamin K is denied by Boyd and Warner [151], who claim that there is a recovery phase after the initial fall in prothrombin caused by dicoumarol They state that the results

of clinical studies are confused by other factors such as withdrawal of the drug and blood transfusion The protective action of vitamin K against the hypoprothrombinæmia produced by dicoumarol is lessened if there is liver damage e g by tumours [374]

Dicoumarol is effective orally parenterally and rectally A single dose will prolong the prothrombin and coagulation times for two to three weeks Its rectal absorption however is irregular and the effect given by this route is uncertain [285] It is more effective in animals in which pyrexia has been induced and care should therefore be exercised when it is used clinically in febrile patients

Dicoumarol produces no changes in the white cell count erythrocyte sedimentation rate percentage hæmoglobin blood sugar icterus index liver function tests non protein nitrogen or in the urine [369]

Clinical Uses and Indications Clinical evidence shows that the carefully controlled and individual administration of dicoumarol is effective in the *prevention of post operative venous thrombosis and pulmonary embolism* [285 286] and in the treatment of venous thrombosis thrombophlebitis [288 292] and the spread of thrombosis after embolism These include cavernous sinus thrombosis puerperal thrombosis [279] mesenteric thrombosis the thrombosis of arteriosclerosis (in cerebral and coronary vessels) and thromboangiitis obliterans and retinal thrombosis Dicoumarol administered prophylactically diminishes the incidence of puerperal thrombophlebitis without increasing the danger of post partum hæmorrhage [279] Brambel and Loker [367] used dicoumarol in post traumatic arteriosclerosis and gangrene due to diabetes and frostbite They concluded that it prevented the extension of established gangrene and prevented it developing in traumatic cases MacLean and Brambel [363] have administered dicoumarol for periods up to six months to patients with central retinal venous occlusion and diabetic degenerative and central serous retinopathies They claim to have improved visual acuity

A committee sponsored by the American Heart Association has investigated the use of anticoagulants in the treatment of coronary thrombosis with myocardial infarction and they conclude from a study of eight hundred cases that the death rate and incidence of thromboembolic phenomena during the six weeks following an attack of the disease was markedly lower in those treated by anticoagulants than in those treated solely by conventional methods [156] Allen and his colleagues [287] have reviewed 2 307 cases of acute vascular thrombosis treated at the Mayo Clinic with dicoumarol They conclude that its use constitutes the greatest contribution to the successful treatment and prevention of intravascular thrombosis and embolism A survey of the prophylactic use of dicoumarol against thromboembolic complications after surgery has been made by Wise Loker and Brambel [387] in 3 300 cases They have shown a statistically significant reduction in the incidence of venous thrombosis and fatal embolism following major abdominopelvic surgery when dicoumarol was used prophylactically This has been confirmed by Borgstrom [395] who states that with early ambulation and dicoumarol the risk is reduced to a quarter

Putnam and his co workers [362] treated forty three patients with disseminated sclerosis with doses of dicoumarol sufficient to raise the prothrombin time to thirty seconds (Quick) for periods varying from six months to four years They were however unable to attribute any improvement to this form of therapy which was given on the assumption that disseminated

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cost of heparin is prohibitive and it must be given intravenously over a long period However dicoumarol cannot be regarded as completely safe as many cases of severe and alarming bleeding have occurred and twenty one fatalities

have been reported [280] The increased prothrombin time following its use persists for several days after its withdrawal Crawford and Nassim [364] describe a case of retinal thrombosis in which there was a delayed response to dicoumarol followed by a severe reaction to the drug The reactions which included hæmatemesis hæmaturia albuminuria and a prolonged increased prothrombin time after the drug was withdrawn necessitated blood transfusion Wasserman and Stats [294] record such well marked individual variations in the response to dicoumarol that they are unable to fix a standard dose Douthwaite [365] and Evans [85] describe cases resistant to dicoumarol Barker and his colleagues [90] at the Mayo Clinic believe that the risk of bleeding after giving dicoumarol has been exaggerated They state that in 1 000 cases on the drug only 3 9 per cent showed evidence of minor hæmorrhage and 2 5 per cent major hæmorrhage Major bleeding was controlled by an intravenous injection of 60 mg of menaphthone bisulphite They state that if the proper contra indications are observed and the dosage carefully regulated the danger of severe bleeding is slight

There are definite contra indications to the use of dicoumarol Absolute contra indications are renal insufficiency purpura hæmorrhagic diathesis prothrombin deficiency e g in cases of jaundice hepatic disease and mal nutrition blood dyscrasia with tendency to bleed and subacute bacterial endocarditis Relative contra indications are ulcerative lesions open wounds bleeding surfaces possibility of operation within two weeks operations on the brain or spinal cord and vomiting due to gastric or intestinal drainage Dicoumarol should not be used as a routine without control after operation because of the danger of hæmorrhage

When given in doses producing an unsafe level of prothrombin in pregnant rabbits dicoumarol is stated to cause death of the fœtus *in utero* [366] care should therefore be exercised when administering the drug in pregnancy Hypoprothrombinæmia can be induced in suckling animals if the mother is given dicoumarol and can be corrected by giving vitamin K to the mother [376] Dicoumarol can be administered to nursing mothers without the hazard of hypoprothrombinæmia in the infant [279 403]

Dicoumarol Therapy Dicoumarol should never be used unless daily and consistently comparable prothrombin time tests are done as it is impossible to predict the dose for any one patient The Quick prothrombin time is often used but there have been objections to this method in patients receiving dicoumarol on account of the numerous variables Hæmorrhage following the administration of dicoumarol is not directly proportional to the concentration of prothrombin [216] The two stage method is the method of choice [222 262] It probably does not matter what technique is used provided the test is carried out with scrupulous attention to detail and the significance of the findings clearly understood The Quick method using viper venom does however appear to give false low clotting times with danger of overdosage [193]

Two hundred to three hundred milligrams of dicoumarol are given daily until the prothrombin time is thirty seconds and reduced to 50 to 100 mg daily if this is between thirty and thirty five seconds The drug is withheld if the prothrombin time is thirty five seconds or more and no more is given until the prothrombin time is down to thirty seconds or less after which the drug is again given continuously in 100 mg doses [363] These prothrombin times are in terms of the Quick or Link Shapiro method [325] It should be remembered that effective prothrombin levels may not be reached until twenty four to forty eight hours and even longer after giving dicoumarol The drug varies in its activity in different people some particularly the

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thirty five seconds several days

or a week after the patient has become ambulatory After stopping treatment

it takes two to ten days for the prothrombin time to return to normal It may take longer than this in cases of liver or kidney damage or in patients with a hæmorrhagic tendency The prothrombin time may be kept elevated for months or even years by dicoumarol [362] Rice and others [401] have given 66 grams of the drug over a period of forty months without any mishap

To get an immediate anti coagulant effect dicoumarol has been combined with heparin the latter being discontinued when the prothrombin time reaches thirty five seconds [296]

Facilities for blood transfusions should always be available Hæmorrhage is treated by a blood transfusion of 500 c c of fresh (not bank) citrated blood repeated several times if necessary as there is a tendency for the prothrombin time to increase again after several hours Large doses of a soluble vitamin K analogue e g 75 to 150 mg are given intravenously every four to twenty four hours [411 415] or vitamin K_1 oxide (180 to 250 mg) [412] or a single dose of 500 mg of vitamin K_1 orally [421] A five per cent emulsion of vitamin K_1 given intravenously in doses of 5 to 20 mg per kilogram of body weight is also effective in overcoming dicoumarol hypoprothrombinæmia [422 425] It is claimed that the protective action of vitamin K is increased by simultaneous administration of vitamins C and P [349]

An analogue of dicoumarol bis 3 3 (4 oxycoumarinyl) ethyl acetate (Tromexan) has recently been claimed to be more potent and safer to administer since it is excreted and destroyed more rapidly [247 424] After withdrawal of the drug the prothrombin level is stated to return to normal in thirty six hours [424] It has a shorter lag period than dicoumarol [423]

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nticoagulants such as

Tromexan Cyclocumarol [279 336 430 431] and phenylindandione which is intermediate in action between heparin and dicoumarol [400] The initial dose of phenylindandione is 200 mg and the maintenance dose 50 to 100 mg

Poisoning has occurred from the unauthorized oral use of Warfarin 3 (α acetonylbenzyl) 4 hydroxycoumarin which has an anticoagulant action and is used as a rodenticide [432]

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CHAPTER XI

VITAMIN P

HISTORY

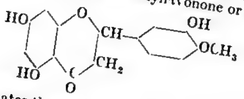
THE existence of vitamin P was postulated in 1936 by Szent Györgyi [1] and his co workers who claimed that extracts of Hungarian red pepper and lemon juice contained a substance which was more effective in the clinical treatment of increased vascular fragility than ascorbic acid. Later it was reported that fractionation of these extracts yielded an active preparation consisting of a flavone or flavone glucoside which was called *citrin* or vitamin P [2]. This was stated to be effective in the treatment of increased capillary permeability in three patients suffering from vascular purpura but it had little effect on four patients with thrombopenic purpura. A moderate effect was observed on the capillary fragility in seven patients suffering from infectious disease myxœdema and diabetes.

Szent Györgyi [2] also stated that citrin decreased the number of hæmorrhages in scorbutic guinea pigs and prolonged the survival period from 28 5 days for the negative control group to forty four days for the animals given 1 mg of citrin daily [3]. It was suggested that the full clinical syndrome of scurvy in the guinea pig was produced by a combined deficiency of ascorbic acid and vitamin P.

Efforts to repeat the observations of Szent Györgyi and his co workers in experimental animals yielded conflicting results. Thus Zilva [5] Moll [6] Hiramatsu [7] Detrick [8] Bensath and Das [9] and McHenry and Perry [33] were unable to confirm them. Zilva administered a vitamin P preparation (0 66 mg hesperidin and 0 33 mg eriodictyol daily) to guinea pigs on a scorbutic diet but he failed to observe any delay in the onset of scurvy or in the time taken for the animals to succumb. He claimed that the administration of a daily dose of 0 1 to 0 2 mg ascorbic acid to such animals produced a condition resembling that obtained by Szent Györgyi and his colleagues by administering a daily dose of vitamin P. Szent Györgyi [10] has since reported his failure to repeat his original experiments upon which the existence of vitamin P was based. More recent work shows that vitamin P does affect capillary fragility in the experimental animal.

CHEMISTRY

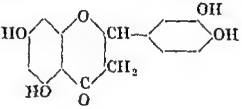
The chemistry of vitamin P was investigated by Szent Györgyi [4] who considered it to consist of mixed crystals of two related flavones the glycoside hesperidin and the glycoside of eriodictyol. According to him the former constitutes the major part of citrin the latter is responsible for the chemical reactivity and yellow colour. Hesperidin is the rhamnoglucose glycoside of 5 7 3 trihydroxy 4 methoxyflavonone or hesperetin —



Mager [58] however states that it is the L rhamnose glycoside. According to Scarborough [65] hesperidin is not vitamin P as there is an active material

in rose hips at least two and a half times more active Moreover, hesperidin is practically insoluble in both water and lipoids

Szent Györgyi believes that eriodictyol glycoside is the glycoside of 5 7 3 4-tetrahydroxy flavonone —



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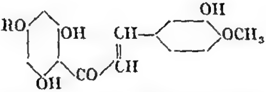
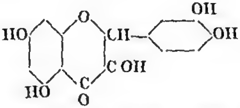


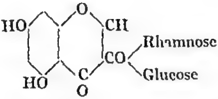
R=a sugar group

and that it is capable of being reversibly transformed by reduction and ring closure to the flavonone glycoside Preliminary experiments showed that the chalcone decreases capillary fragility and prevents hæmorrhage This has not been confirmed by Higby [68]

Robezmeks [69] by means of chromatography showed that citrin contains a quercitrin like substance Quercitrin is 3 5 7 3 4′ pentahydroxy flavonone —



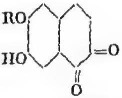
The discovery that rutin, a rhamno glycoside of quercitrin, has vitamin P like activity was made in 1944 by Griffith and his colleagues [66] They first isolated rutin —



Rutin

from tobacco and later found that the best source is buckwheat, some Japanese varieties of which contain as much as six per cent

Epicatechin (p 735) is also a flavonol with a vitamin P like action Esculin —



is a flavone with an activity several hundred times that of citrin

ESTIMATION AND UNITS OF VITAMIN P

A method of estimating vitamin P based on the measurement of capillary resistance has been developed by Bacharach, Coates and Middleton [41] They apply a suction cup with a diameter of 12 mm to the greased and shaven area on the back of a guinea pig, the pressure being gradually reduced by 5 mm stages, and maintained at each stage for three to five seconds The pressure is noted at which petechiæ are first seen when the area under suction is viewed through the cup This is called the critical petechial pressure If this is plotted against the logarithm of the dose of vitamin P administered to the animals, a straight line graph is obtained A similar technique is used by Bourne [48], who uses a suction cup 20 mm in diameter, the pressure being rapidly reduced to the desired level for ten seconds and then released The capillary resistance is then taken as the negative pressure required to produce so many petechiæ that the area under test becomes uniformly reddish purple in colour Animals on a scorbutogenic diet show a steady and significant fall in capillary resistance not corrected by ascorbic acid It is abolished by hesperidin and the citrus glycosides Much confusion in the literature has resulted from the discriminate use of the two types of capillary fragility tests, i e, the positive and negative pressure tests (p 464) There is no significant correlation between the two tests [121]

Bacharach and Coates [50] express the activity of a vitamin P preparation under test in terms of a standard water soluble calcium containing glycosidic complex derived from citrus peel, which is similar to Szent-Gyorgyi's citrin One "provisional unit" (P U) is defined as the activity of 1 mg of this preparation Recrystallized hesperidin had an activity of 100 P.U per gram Bacharach and Coates [64] have prepared a vitamin P standard of potency 160,000 units per 100 gram Black currant concentrates of 1,060,000 units per 100 gram have been obtained

Scarborough [79] using a negative pressure method to assay vitamin P activity has determined the vitamin P content of a number of foodstuffs He uses a water-soluble preparation obtained from orange pulp and peel by a modification of Szent Gyorgyi's method [10] as a standard This appears to have the same activity as Bacharach and Coates' preparation, 100 mg of it being equivalent to 100 units of "vitamin P activity" or to 1 gram hesperidin or 150 mg of Szent Gyorgyi's citrin

Field and Rekers [113] have devised a "screening" test for substances with vitamin P activity depending upon protection against irradiation hæmorrhage (p 743) Other suggested methods of assay depend on the

DISTRIBUTION OF VITAMIN P IN FOODSTUFFS

Fruits are the richest source of vitamin P, followed by green leaves. There is very little vitamin P in roots and seeds, although there is an increase in the latter on germination Little vitamin P is lost in the processing of commercial fruit concentrates and syrups, but some occurs on storage Although vitamin P and C are associated in fruits and vegetables there is no correlation between absorbic acid content and vitamin P activity in the same food [64], nor between vitamin P and the anthocyanins

There is complete loss of potency if the food is boiled in air If a solution containing potent vitamin P be kept in the light but without contact with air, and in a cool place its activity is maintained for one to two months. At room temperature a preparation of blackcurrant with a potency of 600 units falls to about 200–300 units in three months, and to 150–200 units in six months, while after nine months the potency is negligible [79]

The figures in the following table, taken from Bacharach [41] and Scarborough [79] are very approximate, the authors themselves admitting that they should be accepted with reserve

VITAMIN P CONTENT OF FRUITS AND VEGETABLES

Food	Description	Part Tested	Vitamin P Content in Units per 100 grams
Apple	Bramley's seedling	Fruit	60
Apricot	Dried	Whole fruit	75 100
Beetroot	—	Root	15
Bilberry	—	Whole fruit	100
Blackberry	—	Fruit	60–100
Blackcurrant	Puree = 65%	Raw fruit	200 500
"	Fresh	Fruit	75
Cabbage	Spring (April)	Leaves	60
"	Summer (October)	"	100
Carrot	April crop	Root	10
"	August crop	"	40
Cauliflower	—	Flower	40
Cherry	Black	Fruit	60–100
"	White	"	50
Dandelion	—	Leaves	30
Dock	—	"	20
Grape	Black	Whole fruit	500–1 000
"	White	"	500–1 000
Grapefruit	—	—	100
Lemon	Fruit	Peel	500
"	"	Juice	450 750
Lentil	—	Seed	0
Lettuce	Round (May)	Leaves	80
"	" (September)	"	100
Maple pea	Dried	Seed	10
Orange	Fruit	Whole fruit	300–500
"	"	Juice	300–600
"	"	Peel	200–300
Parsley	—	Leaves	130
Parsnip	—	Root	40
Pea	Dried	Seed	40
"	Germinated	"	80
" maple	Dried	"	10
" "	Germinated	"	10
Plum	Blue	Fruit	50 200
Prune	—	Whole fruit	300–400
Potato	Old (April)	Tuber	25
"	New (July)	"	40
Rhubarb	—	Stalks	20
Rose hips	—	Fruit	240–680
" " syrup	Fresh	"	350–500
" " "	Commercial	"	0–100
Rowan	—	"	300
Spinach	—	Leaves	130
Swede	—	Root	20
Tomato	—	Fruit	50 70
Turnip	—	Root	20–30
Walnut	—	Kernel	100
Watercress	April crop	Shoots	10
"	October crop	"	70
Concentrates			
Water soluble concentrate	—	From blackcurrant	300 000
"	—	From whole orange	100 000
Citrin	From lemon by Szent Gyorgyi's method	From whole lemon	65 000
"Hipexa"	—	From rose hips	20 000 30 000
Hesperidin	—	M P 265° 266°	9 500–10 000
Hesperetin	—	M P 223°–224°	2 500 5 000
Rutin		—	5 000

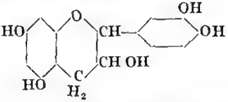
HUMAN REQUIREMENT OF VITAMIN P

Very little is known about the vitamin P requirements of man From dietary studies Glazebrook, Scarborough and Wokes [75] concluded the daily requirement to be not less than 33 units daily, and possibly considerably
intake of vitamin P needed to
two scorbutic subjects was of
nt in 50 to 100 ml of orange
juice and would be provided by three grams of hesperidin This is of interest as Gothlin, fourteen years earlier, concluded that 50 70 ml of orange juice was the minimum daily dose to protect against scurvy in man (p 441)

Seasonal variations in the human intake of vitamin P occur In a group of boys studied by Glazebrook Scarborough and Wokes [75] the vitamin P increased from 7 units daily between February and March to 15 units in August and September This was probably below the normal daily requirements of vitamin P

THEORY OF ACTION OF VITAMIN P

In 1941 Lavollay and his colleagues in France [52] put forward the view that vitamin P controls capillary resistance and permeability by retarding the oxidation of adrenaline, sympathin or one of their metabolic products such as adrenochrome These are then able to exert a vasoconstrictor effect on the capillaries and diminish capillary fragility They tested D catechin, or D 3 5 7 3′ 4′ pentahydroxy benzodihydropyran and found this to be inactive, but further investigations led to the isolation of D epicatechin a related compound, which was found to be extremely active Wilson [81] confirmed Lavollay s observation that flavonols such as rutin prolong the effect of adrenaline in isolated organ experiments, and it has been shown that rutin diminishes the amount of adrenaline required to produce a vasoconstrictor effect on blood vessels [138]

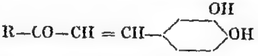


Epicatechin

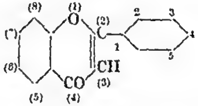
According to Haley, Clark and Geissman [70] the catechins are highly active vasoconstrictors in doses of 0 0001 microgram or less They have tested the effect of substances with a vitamin P like activity topically on mammalian capillary bed preparations, observing microscopically vasomotor responses of the vessels They found that rutin and hesperidin were inert in the concentration used, although the catechins including the D catechin and L epicatechin were highly active Lavollay and his co workers found D epicatechin the most active vitamin P like compound It is possible of course that intravascular contact with the compounds under test may differ from the topical effects The theory of Lavollay and his followers and their method of assay of vitamin P like substances is not accepted by some workers [73] Using adrenergic blocking agents Schiller [141] was able to show that although rutin prolongs the vasoconstrictor action of adrenaline and noradrenaline it also has a strong cutaneous vasoconstrictor action of its own , adrenaline and rutin do not compete for the same receptors

It is clear that " vitamin P " is not an entity in the sense that aneurine,
quercitrin
ons possess
numerous
compounds for their potentiating effect on the action of adrenaline on excised

smooth muscle and conclude that for high activity the following structure is necessary —



They could not, however, correlate this potentiating effect on adrenaline with the effect on capillary fragility and reject it as a method of assaying "vitamin P" Wilson and De Eds [72] tested a number of flavonols and flavonones, both natural and synthetic for their ability to protect adrenaline from destruction, using an isolated strip of intestine as the test object Using the notation indicated in the general formula of the flavones given below, Wilson and De Eds conclude that a glycosidal linkage



on C – 7 increases, while methoxylation (OCH_3) of this carbon atom decreases activity, a double bond between C – 2 and C – 3 increases activity, and two free hydroxyl groups ortho to each other on the phenyl ring also increase activity

One of the difficulties encountered in evaluating the vitamin P potency of

negative pressure methods, and the effects of age, sex, season, time of day, emotion and menstruation further complicate the picture The role of adrenaline in controlling capillary permeability, as suggested by Lavollay, is by no means clear since Clark [74] has shown that adrenaline actually causes dilatation of muscle capillaries Changes in capillary permeability have been followed by observing changes in cutaneous lymph flow by noting the spread of dyes, such as Evans blue, after injection into the antecubital space The application to the skin of an irritant, such as chloroform, or the intracutaneous injection of histamine, results in the accumulation of intra venously injected trypan blue in the areas of inflammation [76] Ambrose and De Eds [77] have used this technique as a method of estimating capillary fragility and of assaying the vitamin potency of various flavones, the method is only applicable when the substance under test can be injected, and a com plicating factor is that the rate of escape of the dye from cutaneous capillaries is probably influenced by changes in the blood pressure Wilson and his co workers [81] claim that rutin inhibits the extravasation of dye given intravenously into histamine wheals, although this cannot be confirmed by Clark and MacKay [128] Bohr, McIvor and Rinehart [80] criticize the dye technique and ascribe the changes produced by the flavones to a decrease in capillary blood flow in the skin rather than to changes in capillary permeability The potency of flavonoid compounds has also been estimated by an air blast method, which ruptures the lung capillaries [131], the method does not give statistically significant results [132]

In scorbutic human beings the positive pressure test for measuring capillary resistance (p 464) fails to show any change after the administration of vitamin P preparations, there is a slight improvement using the negative pressure method [78] The capillary resistance of a group of 100 allergic children was studied by Rapaport and Klein [29] Twelve with a low capillary resistance were treated with 100 to 150 mg of a vitamin P preparation daily

for six months when the capillary resistance became normal The capillary resistance of normal persons is not influenced by vitamin P [63]

It has been suggested by Levitan [67] that hyaluronidase may be a factor in controlling tissue and capillary permeability and that hyaluronidase is inhibited by vitamin P like substances He states that rutin markedly inhibits the spreading activity of intradermally injected hyaluronidase Rodney and her co workers [127] observed that some flavonoid compounds inhibited the action of hyaluronidase but only those capable of orthoquinone formation They did not regard the effect as specific Elster [85] failed to observe any inhibiting effect of rutin on the increased diffusion across capillary membranes caused by hyaluronidase Levitan [86] believes that the effect of vitamin P on the capillaries is due to a non specific increase in the resistance of the connective tissue ground substance in the pericapillary sheath In vitamin P deficiency induced by feeding an antivitamin P factor the permeability of connective tissue was increased this was partially reversed by aesculin a flavonol but not by ascorbic acid

PHYSIOLOGY AND PHARMACOLOGY OF VITAMIN P

The work of Zacho [11] Bacharach [41 50 64] and Bourne [48] suggests that vitamin P like compounds play a part in the control of capillary permeability and resistance in the experimental animal The low capillary fragility of scorbutic guinea pigs rises after giving ascorbic acid but still keeps at a subnormal level When citrin is added to the diet however the capillary fragility reaches normal levels The intestinal hæmorrhages of scorbutic animals also clear up when vitamin P is added to the diet Rusznyak and Benko [31] found that lowered capillary resistance in rats could be raised to normal levels in ten to fourteen days by subcutaneous injections of 3 to 4 mg of citrin Todhunter and his co workers [15] report that scorbutic guinea pigs receiving supplements of lemon juice show fewer hæmorrhages than do animals receiving equivalent amounts of synthetic ascorbic acid They observed that on a scorbutic diet reinforced by synthetic ascorbic acid the capillary fragility decreased considerably but only rose to normal levels or above after the administration of vitamin P or citrin

Sokoloff and Redd [73] induced scurvy in animals by means of glucoascorbic acid an ascorbic acid antagonist and produced complete recovery by treatment with ascorbic acid and vitamin P Crampton and Lloyd [82] using the odontoblast method for the assay of ascorb c acid state that rutin enhances the biological effect of ascorbic acid Cotereau and others have

from citrus fruits can diminish increased capillary fragility due to leukotaxine a substance obtained from inflammatory exudates and bacterial polysaccharides [142 143]

Citrin and the flavones with vitamin P activity cause a slight fall in blood pressure when administered intravenously a dose of 100 mg of citrin causes a fall of 10 to 15 mm after about three to four seconds [89] This fall is apparently due to vasodilatation On the other hand the catechins produce capillary vasoconstriction [70] In the scorbutic guinea p g there is a twenty to thirty per cent increase in the reticulocytes within a few days of giving citr P

tior

been confirmed by Martin and Swayne [88] for rutin and D catechin but not

for hesperidin Clark [89] could not confirm the protective action of rutin against dicoumarol D Catechin reduces the bleeding time in man from a mean of seven and a half minutes to one and a half minutes [94]

Rutin, hesperidin and other flavonoids have been stated to give some protection to guinea pigs from anaphylactic and histamine shock although the effect is not spectacular [47, 81, 140] and is even denied by some workers [124 128] Wilson, Mortarotti and De Eds [91] suggest the protection against histamine shock is due to the potentiation of adrenaline in the tissues by the *flavonols There is some evidence that citrin protects guinea pigs against the action of diphtheria toxin, although it is not as effective as ascorbic acid* [88] Flavones have a detoxicating effect on benzene phenol [49] and thiocyanates [88] and with ascorbic acid protect against the toxic effects of

id epimerized
ition of hista
[128] Rutin

prevents the leucocytosis that can be produced by *irritating the skin* [136] and the anæmia and leucocytosis following splenectomy in the albino rat [139]

Vitamin P has a moderate inhibitory effect on the growth of mouse sarcoma and carcinoma [146] It prolongs the life of adrenalectomized rats [146]

Certain flavonoids inhibit the action of choline acetylase *in vitro* [130] Whether this occurs *in vivo* and the significance of effect are not known

Hartzell and Stone [54] have shown that vitamin P, unlike ascorbic acid plays no part in the healing of wounds

Vitamin P is absorbed when given orally, and it is active when administered parenterally It is excreted in the urine [87] *Apparently vitamin P is not* stored to any appreciable extent in the tissues [36] After the administration of test doses of 50 to 100 mg of citrin or eriodictyol intravenously about fifty per cent is excreted in the urine [37], twenty per cent of a dose of epicatechin can be recovered from the urine [90] Saturation of the tissues is reached in normal persons in from two to six days, but in disease a much longer period is required In vascular purpura saturation cannot be attained [37]

Substances with a vitamin P action such as hesperidin and rutin are virtually *non toxic* even in large doses *e g*, 15 grams daily [92 93] Patients have tolerated 120 mg rutin for nine months without any side effects [95] The growth rate of rats is uninfluenced by incorporating one per cent rutin in their diet [93]

HUMAN VITAMIN P DEFICIENCY

A number of clinical studies suggest that vitamin P is an essential factor in human nutrition The observations of Scarborough [12–14 65] appear to establish with some certainty the existence of a dietary factor essential for the maintenance of capillary resistance in human beings He observed an increased capillary fragility in a number of patients suffering from multiple vitamin deficiencies of varying degrees of severity In two patients with scurvy the daily administration of 3 grams of hesperidin and a rose hip preparation containing 550 units produced a significant rise in capillary resistance [65] Previous treatment with ascorbic acid had produced a fall Scarborough observed that orange and lemon juice and extracts made from them produced an increase in the capillary resistance even when ascorbic acid had failed to produce any effect He concluded that at least two forms of subcutaneous bleeding may develop as the result of nutritional deficiency in man [14] One form due to ascorbic acid deficiency is characterized by the

bleeding is also common Vitamin P does not control
which are only arrested by large doses (500 mg) of ascorbic acid Scarborough also reported that vitamin P has no effect on the other important manifesta

tions of the scorbutic state, but apparently it can increase the capillary resistance of scorbutic subjects either before or after treatment with ascorbic acid

Cameron and Mills [55] gave vitamin P but not ascorbic acid to a patient with classic scurvy The hæmorrhagic features promptly disappeared although the other symptoms of the disease were unaffected until ascorbic acid was given Ambrose and De Eds [116] state that supplements of rutin and ascorbic acid prolong the life of scorbutic guinea pigs longer than either substance alone Lazarus, Munro and Bell [96] do not consider the association of capillary resistance and scurvy to be clear cut In a series of fourteen cases the capillary resistance was within normal limits There was no change in capillary strength after treatment with either vitamin P or ascorbic acid Although the patients were cured clinically they consider that scurvy is more liable to develop in subjects who already have some form of capillary weakness, which is probably present in a small proportion of the population, e g, they noted five medical students with a high capillary fragility that did not respond to ascorbic acid or vitamin P

Scarborough believes that a deficiency of vitamin P may exist in man even after dosage with large quantities of ascorbic acid The clinical manifestations of vitamin P deficiency that he describes include pains in the legs on exertion, pain across the shoulders, weakness, lassitude and fatigue It is invariably associated with a much decreased capillary resistance and may be characterized by the development of spontaneous petechial hæmorrhages, especially in areas exposed to pressure (e g of tight clothes) It has not been found to be accompanied by any hæmatological abnormality, and it responds to treatment with vitamin P (50 mg doses) The hæmorrhages developing as a result of vitamin P deficiency are always small (petechiæ) and take place in the skin They are often circumpilar Hæmorrhage is more severe in parts exposed to the pressure of clothing and in the legs because of the higher venous pressure in the latter Scarborough considers that some forms of purpura may have a nutritional basis, although he states that vitamin P is ineffective in its treatment [56] He has described an experimentally induced clinical syndrome in two human subjects attributable to a deficiency of vitamin P [32] The major features of this syndrome are petechial bleeding, low capillary resistance, and a slightly prolonged bleeding time.

CLINICAL USES OF VITAMIN P

Various flavones with vitamin P activity have been used clinically, e g, citrin (20 to 60 mg daily), hesperidin (0 25 to 1 gm daily), and rutin (60 to 180 mg daily) The latter is readily obtained from buckwheat and is now the preparation that is generally used Preparations of lemon of unknown potency have also been employed so that caution is needed in interpreting results Vitamin P preparations have been used mainly in those conditions with decreased capillary resistance These include diabetes, hypertension, rheumatic fever, allergic conditions, bacterial toxæmias and toxic manifestations from drugs Unfortunately much of the work has been done on small numbers and not controlled statistically, and no standardized method has been used for determining capillary resistance Many of the statements made are suggestive but not conclusive

Vascular Purpura There have been numerous reports on the treatment of purpura with vitamin P preparations It has not been established that any form of purpura is primarily due to an increased fragility of the capillary walls Scarborough [27] points out that the presence of extravascular blood, either in the tissues or in the alimentary canal, markedly increases the capillary resistance and so makes petechial counts as a measure of the latter very inaccurate Thus after the development of a purpuric eruption resulting in the extravasation of more than 4 ml of blood into the tissues, the capillary

resistance will be high for two to four days and the individual may show a decreased tendency to the development of further purpuric spots during this period Failure to recognize this means that entirely fallacious conclusions may be drawn from observations on the therapeutic value of vitamin P in purpura Some of the conflicting results may also be due to differences of technique used by various workers It is known for example that there is no correlation between the negative and positive pressure methods (p 464) for *measuring capillary fragility* [63, 121] Kugelmass [16] claims to have satis factorily treated four cases of purpura of nutritional, allergic and infectious origin with vitamin P in doses of 150 mg daily by mouth A case of purpura following scarlet fever showed improvement, but traumatic conjunctival petechiæ, resulting from attacks of whooping cough and epilepsy showed no change, nor did purpura caused by the pressure of plaster casts Miller [28] records a case of purpura developing during the convalescent stage of measles that responded to treatment with vitamin P in eight days In a single case such as this natural recovery cannot be excluded

Jersild [17] treated a case of Schonlein Henoch purpura refractory to ascorbic acid, with 50 mg of a vitamin P prep[illegible] but reappeared when this was withdrawn, an[illegible] vitamin was re administered Hiramatsu [illegible] Schonlein Henoch's purpura one of purpura following rheumatic fever, and one case of purpura simplex with vitamin P All showed an increased capillary resistance, the effect being potentiated by ascorbic acid Vacek [34] states that in thrombopenia vitamin P causes the disappearance of petechiæ, although it does not affect the platelet count Scarborough [32] records two cases of *senile purpura that responded to vitamin* P

These observations have not been confirmed by others Vaughan [98] Rudy [62] and Franke [26] failed to observe any improvement in cases of purpura treated with vitamin P Davis [97] as a result of analysing 1,200 cases of Schonlein Henoch purpura concluded that neither vitamin P nor ascorbic acid were of any value in treatment Madison and Pohle [43] examined the effect of rutin in fourteen cases of purpura—six allergic two [illegible] associated [illegible] the four [illegible] were not convincing and in the remainder they were questionable Vitamin P does not affect the thrombocyte count in purpura and analysis of the published work reveals that vitamin P is of doubtful value in raising the capillary resistance unless lowered by infection and nutritional deficiency, the results in allergic purpura are not convincing

Several investigators have reported that purpura due to drug toxæmia is favourably affected by vitamin P Scarborough and Stewart [12] showed that the diminished capillary resistance that sometimes occurs as a complication of arsenic and bismuth therapy in the treatme[illegible] can be checked by giving hesperidin in doses of 1 gram daily [illegible] also observed that the toxic erythema an[illegible] from the treatment of syphilis with arse[illegible] *capillary resistance, and that t*[illegible] vitamin P Gorric [20] reported a case [illegible] injections of neoarsphenamine [illegible] gram of vitamin P daily Capillary damage is more likely to occur in the [illegible] the arsenicals Goldstein Stolman [illegible] ed by administering [illegible] the arsenical They administered 10 to 30 mg per kilogram of hesperidin methyl chalcone daily for seven days to rabbits before treatment with toxic doses of mapharsen In the treated group the survival rate was ninety per cent in the untreated group fifty seven per cent Friend and Ivy [59] observed that hesperidin

methyl chalcone and ascorbic acid had a similar protective action against the toxic effects of another arsenical, dichlorophenarsine

Hæmorrhagic Telangiectasia Hereditary hæmorrhagic telangiectasia, or Rendu Osler Weber disease, also known as the Sturge Weber syndrome, is a rare condition characterized by multiple telangiectasia, hereditary transmission, and widespread hæmorrhage The latter may result in epistaxis, hæmatemesis, melæna, hæmaturia and small cerebral hæmorrhages which may cause paralysis Telangiectases can appear in the skin, mouth, alimentary canal and brain Kushlan [18] has reviewed 1,000 cases in 175 families and records the treatment of one case with 40 mg of rutin daily after five blood transfusions had failed to stop a severe hæmatemesis, epistaxis and bleeding gums Improvement was stated to have occurred within twenty four hours of administering rutin Cope and Grover [114] also record improvement in a case treated with 120 mg of rutin daily Petch [119] and Glass [135] failed to observe any benefit in cases treated with rutin, the cases mentioned by Glass showed no change in capillary fragility

Nephritis and Hæmaturia Vitamin P has been used in the treatment of hæmorrhagic nephritis and hæmaturia due to food allergy, drug idiosyncrasy and bacterial toxins [1, 20, 21] One observer however administered vitamin P to seven patients with hæmorrhagic nephritis and obtained results that were no better than those obtained by rest in bed, careful nursing and dieting [22] Gorrie [20] reports the case of a patient with acute hæmaturia occurring after several injections of neoarsphenamine the hæmaturia ceased forty eight hours after commencing treatment with vitamin P Raunert [23] treated thirty cases of hæmaturia due to nephritis renal tumours, cystoscopy and prostatectomy with 50 to 100 mg of vitamin P every four hours and claimed that the hæmaturia ceased within a few hours Hæmorrhage in a case of hæmaturia due to congenital polycystic disease of the kidneys is stated to have stopped after administering rutin [99]

Hypertension Rutin can prevent experimentally produced malignant hypertension in dogs [145] Griffith and Lindauer [100] observed that in eighteen per cent of 265 cases of hypertension there was a decrease in capillary resistance, which they supposed predisposed to retinal and cerebral hæmorrhage, capillary resistance was further decreased by administering potassium thiocyanate to correct the hypertension In a later communication Griffith [101] reported on a group of 1,600 hypertensive subjects Nineteen per cent showed increased capillary fragility, as measured by Gothlin's test (p 464) modified by Griffith and Lindauer [52], and an additional eleven per cent increased capillary permeability as evidenced by increased cutaneous lymphatic flow measured by the patent blue method of McMaster Patent blue is a dye which when injected intracutaneously is absorbed through the lymphatics which it colours and renders visible According to Griffith the incidence of cerebral and retinal hæmorrhage was greater in patients with capillary defects (nine per cent) than in the controls (two per cent) Rutin in a dose of 20 mg three times daily was given to the patients for periods up to four years Capillary tests became normal in seventy five per cent of those treated, they remained constantly or intermittently abnormal in the remaining twenty five per cent In the majority of cases rutin had no beneficial effect in lowering blood pressure The incidence of cerebral and retinal hæmorrhage was, however, reduced to about that of the normal group The administration of rutin enabled thiocyanate to be given without further increase of capillary fragility It must be pointed out that vitamin P preparations do not have any direct effect on hypertension Griffith's observations have been repeated although on a much smaller number of subjects by Zfass [52] and Shanno [51] who reports that rutin is effective in controlling pulmonary hæmorrhage As only two cases of the latter were treated and the condition often ceases spontaneously it is difficult to make any comment on this Barishaw [122] states that a combination of hesperidin

and ascorbic acid reduces abnormal capillary fragility in hypertensive patients. It is claimed that rutin reduces increased capillary fragility in hypertension occurring in pregnancy [134]. Soloff and Bello [102] did not observe any change in the Rumpel-Leede test for capillary fragility after administering rutin to hypertensive patients, nor did they observe any correlation between retinal hæmorrhage and a positive Rumpel-Leede test.

Glaucoma and Retinal Hæmorrhage. In 1942 Schmidt and Saubermann [57] reported that intraocular pressure in hydrophthalmic rabbits is reduced by intravenous injection of vitamin P. According to Stocker rutin has had no effect on the permeability of the blood-aqueous barrier in rabbits [103]. The same observer administered rutin in doses of 20 mg. three times a day to twenty-six patients with glaucoma for an average period of eight months. In seventeen the intraocular tension fell; in four the results were equivocal and in five there was no change. Shanno, Griffiths and Lamotte [104] consider that some cases of retinal hæmorrhage may be due to a capillary weakness throughout the body. From a study of seventy-nine patients they state that there is an abnormal response to capillary fragility and permeability tests in most subjects with recurrent retinal hæmorrhage. The administration of 60 mg. of rutin daily resulted in a return of capillary fragility and permeability to normal levels in fifty to seventy per cent. of the patients investigated and a reduction in the subsequent incidence of retinal hæmorrhage in many of these. Mathewson [61] describes an extensive case of retinal hæmorrhage, associated with hæmorrhage in other parts of the body, that ceased on administering vitamin P. Another case of recurrent hæmorrhage into the anterior chamber after cataract extraction responded to treatment with vitamin P. Wolffe and Danish [35], however, report the occurrence of subconjunctival hæmorrhage in two out of sixty patients receiving rutin in doses up to 150 mg. daily.

Rheumatic Fever and Rheumatoid Arthritis. Rinehart [105] treated thirty-nine cases of rheumatic fever with vitamin P and claims that it reduced the sedimentation rate within a month. Warter and his co-workers [106] state that hesperidin reduces abnormal capillary fragility in patients with rheumatoid arthritis, but they do not report on its effect on the general condition of the patients. Similar observations were made by Ra[illegible] using hesperidin and ascorbic acid. Rudy and oth[illegible] in the capillary fragility of patients with rhe[illegible] vitamin P preparations.

Diabetes. Many diabetics with [illegible] with hæmorrhagic retinitis show increased capillary fragility, which, it has been claimed can be reduced to within normal levels by administering rutin in doses of 20 to 60 mg. three times a day or by hesperidin [137]. Increased capillary fragility occurs in diabetic patients as an early evidence of generalized arterial disease and is closely correlated with the development of retinitis and later nephritis. There is no evidence that rutin alters the course of the disease in diabetic retinitis [111, 144]. Some workers admit that there are so many variable factors influencing diabetic retinitis that any improvement in the small numbers studied cannot be attributed to rutin or ascorbic acid therapy [111]. Rudy and his co-workers [62], while they confirm the increased capillary fragility in diabetes, state that it is uninfluenced by vitamin P therapy. Palmer and his colleagues [147] consider that the results obtained with rutin in the treatment of diabetic retinitis were no better than would be expected from good diabetic control with diet and insulin.

Skin Diseases. Vitamin P has been used in the treatment of some skin diseases with inconclusive results. It has been used in the treatment of eczema [24, 44] and psoriasis [30]. Goldfarb [30] claimed to have obtained improvement in thirty out of forty-five cases of psoriasis treated with citrin preparations made from lemon, but Niedelman and Horoschak [112] obtained no benefit from giving hesperidin and ascorbic acid in this condition.

Irradiation Sickness Exposure to heavy but sublethal doses of X rays or other forms of radiation causes purpura with severe and often fatal hæmorrhage from mucous membranes On the assumption that the intractable capillary oozing is due to excessive capillary fragility Field and Rekers [113] administered a number of flavonols to dogs s... ...s
Rutin was first tried in a large dose ... X
to stop the leuconen...
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Similar obse... ...ve been made ... the most marked ... signs and post mortem ...ven per
In Field and Reker s dogs the flavonols had little effect unles they were given before exposure to irradiation Other flavonols which appeared to be approximately equally as active as rutin were hesperidin epimerized D catechin and homoeriodictyol but quercetrin quercetin naringin and hesperidin methyl chalcone were inactive Ascorbic acid alone was also inactive but with quercetin did afford protection to ten per cent of the dogs Field and Rekers propose to use the protective effect against irradiated dogs as a screening test for vitamin P activity Other workers have failed to observe any beneficial effect in animals submitted to X radiation [120 148]

Mathewson [61] reported a case of myeloma treated by X ray spray therapy which caused an acute hæmorrhagic diathesis This was checked after the administration of vitamin P Madison and Pohle [43] describe four advanced cases of cancer treated by irradiation that benefited by the administration of rutin Sokoloff Eddy and Redd [142] administered 300 to 600 mg of a flavonoid preparation from citrus fruits to ninety two patients receiving radiation therapy skin erythema was diminished considerably but the incidence of nausea and vomiting was unchanged

The subject requires further investigation in view of the irradiation effects likely to be experienced in atomic warfare

Frostbite Fuhrman [115] induced frostbite artificially in guinea pigs and states that if they are previously treated with rutin they suffer less tissue damage Vitamin P has not been used clinically for frostbite

Allergic and Vasomotor Rhinitis On the assumption of Lavollay that vitamin P retards the oxidation of adrenaline the antagonist of histamine Saylor [123] treated allergic and vasomotor rhinitis with hesperidin chalcone in doses of 100 to 600 mg daily He claims that thirty five per cent of ninety nine patients obtained complete relief from symptoms thirty four per cent obtained partial relief and twenty five per cent experienced no relief Five per cent showed side reactions such as urticaria nausea and aggravation of bronchial asthma

Clark and MacKay [133] have assembled evidence to show that these effects of flavonoid compounds are not due to any vitamin like action or that they exert any specific chemical or therapeutic effects They have shown that the compounds are not absorbed from the human gut and are in fact destroyed either by enzymes or organisms in the stools When injected they may cause a decrease in adrenal ascorbic acid and thus may elicit a non specific stress or alarm reaction (Selye), which may explain some of the physiological and pharmacological effects

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